THE ROLE OF DOMESTIC ANIMALS IN THE SMALL-SCALE ECOLOGY OF *Triatoma infestans*, A VECTOR OF CHAGAS DISEASE

By

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Dissertation Abstract

This dissertation investigates the associations between domestic animals and the ecology of *Triatoma infestans*, a vector of Chagas disease. The ecology of *Triatoma infestans* in relation to domestic animals has been studied in other areas, but it is context dependent and the approaches might differ. This dissertation combines field observations and experimental data, and uses concepts from epidemiology, entomology, and ecology to understand the interactions between domestic animals, *Triatoma infestans* and *Trypanosoma cruzi*, the parasite the causes Chagas disease.

The first aim uses a novel approach to analyze the influence of domestic animal species and animal corral construction materials on the presence and abundance of *Triatoma infestans*. This analysis combines both, domestic animal species and construction materials, to define *ecotopes*, the smallest ecologically distinct landscape unit. These ecotopes might increase or reduce the odds of finding *T. infestans* and their abundance. We found that small mammals (guinea pigs and rabbits) living in enclosures built with stacked stones or bricks increases the odds of finding insect vectors and of finding more abundant colonies. Other animal species raised in enclosures built with wire mesh showed a protective effect against harboring insect vectors.

The second aim proposes to use dogs as animal sentinels to determine areas of transmission of *Trypanosoma cruzi*. We analyzed data from a cross sectional entomological survey and a canine serological survey and found a strong spatial association between seropositive dogs and parasite-carrying vector colonies. In addition, increasing age seroprevalence curves and behavioral features of dogs support the hypothesis that dogs can be good animal sentinels for *T. cruzi* transmission. This section of the dissertation also discusses feasibility of dog sentinels and proposes ways to integrate this approach with current health programs.

Finally, the third aim examines the influence of the population dynamics of domestic animals on *Triatoma infestans* dispersion. We created a laboratory experiment to explore and characterize the host-seeking behavior of *T. infestans* and its dispersion under the constant presence of hosts and after these hosts are removed. We found that there was an important level of random dispersion in the constant presence of hosts, but population dynamics of hosts had a strong influence on vector dispersion. After host removal, we found spatial patterns of dispersion that suggest that empty animal enclosures remain attractive for vectors, and most importantly, vector activity increased significantly after hosts were removed which might indicate increased risk for humans in infested areas where animal populations fluctuate.

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1. Chagas disease, *Trypanosoma cruzi*, and *Triatoma infestans*

In Latin America, approximately 7 to 8 million people are estimated to be infected with *Trypanosoma cruzi* [1], the causative parasite of Chagas disease, and more than 100 million people are at risk of being infected [2]. Most people infected with *T. cruzi* have mild or no symptoms during the acute phase of the disease (roughly within the first 30 days of infection), and pass into the chronic phase of infection without being detected [3,4]. Once in the chronic phase, most infected individuals show no signs or symptoms and are considered to have the indeterminate form of the disease. However, it is estimated that 20 to 30% of those infected will develop cardiac or digestive forms of Chagas disease, usually decades after initial infection [3,4]. This clinically manifest form of the disease cannot be reversed and is often fatal [3-5].

1.1. Life cycle of the parasite and vector hosts

The life cycle of the *Trypanosoma cruzi* parasite involves vectors, humans, and animal reservoirs [6] and is transmitted between hosts and reservoirs by triatomine vectors. There are many triatomine species that can transmit *T. cruzi*, but only some are of medical importance because their feeding or living habits involve proximity to humans [7]. In the southern cone of South America, *Triatoma* *infestans* is the most important insect vector of *T. cruzi*. *T. infestans* is a nocturnal blood-sucking insect that feeds on humans and other mammals, as well as on birds [7]. When the infected vector feeds on a host, it defecates and release infective forms of *T. cruzi* with the intestinal contents [8]. The parasite contained in the excreted feces can enter the bloodstream through any break in the skin or through the oral or ocular mucosa [9]. The insect tends to form colonies comprised of adult males, adult females, and nymphs of different developmental stages (from first to fifth instar). The adult insect is about 1 inch long and resembles a flat cockroach when it has not eaten, becoming rounded and bloated after a blood meal, which usually takes about 20 minutes. Only adults have wings but they are not efficient for flying. *T. infestans* live within or in the proximity of human homes. They seek crevices, cracks, and holes in walls, roofs or other parts of a house, that can give them protection and appropriate environmental conditions to reproduce.

1.2. Strategies of Chagas disease control

Strategies to control the disease include blood screening to reduce transmission through blood or organ donation, screening of pregnant women in endemic areas to detect congenital transmission and treatment of newborns, improving housing to reduce vector infestation in homes, and conducting insecticide residual campaigns [10]. The application of insecticide has proven to be the most cost effective strategy to control the vector [11].

The insecticide spraying campaigns in endemic areas are usually conducted by governmental agencies and their main goal is to reduce the vector population to such a level that vectorial transmission would be halted. Resources for these campaigns are limited and efforts will wane if funding for control programs are reduced, as was the case in Brazil in 1986 when the 600 members of the Chagas control staff were relocated to the mosquito control program [12].

1.3. Chagas disease in Arequipa, Peru

In Arequipa, located in the Andean area of Peru, *T. infestans* is the sole vector of *T. cruzi* and vector-borne transmission of Chagas disease is an important public health problem [13-15]. Historically, the disease had been reported as a rural disease affecting mainly the valleys around the city of Arequipa where housing was rustic and agriculture was the main economic activity of the Andean state of Arequipa. In La Joya district we studied 4 communities in 2008 and found a human seroprevalence of 13.4% (n=1,333). Some datasets collected from that study were used for the present manuscript.

During recent decades, mining and other business activities have grown, and the ensuing economic prosperity in the city has caused migration from the rural areas of the state into the cities, primarily the capital city, Arequipa. Recently, the disease has been detected in the heart of the city [13,15], underscoring an urbanization process of Chagas disease that has been seen in other areas of Latin

America where cities are growing [16]. Human Chagas disease in urban Arequipa, has been detected especially in the periurban outskirts [17]. Bayer and Hunter in 2009 described the patterns of migration and settlement in these communities in Arequipa [18]. They suggest that the practice of city shantytown residents of migrating seasonally to pursue temporary agricultural work in the rural valleys where *T. infestans* is present may contribute to the growth of Chagas disease in the city of Arequipa when these migrant workers return to their urban homes.

1.4. Guinea pig husbandry in Peru

Domesticated guinea pigs are a popular source of animal protein for the Andean highland inhabitants. Guinea pigs were domesticated over 7,000 years ago [19] and are an important part of the economy and folklore of the Andes [20,21]. These animals are typically fed with fresh pastures, mainly alfalfa, and their diet is sometimes complemented with kitchen leftovers [22,23]. They are commonly raised in small groups of a few specimens in backyards, corrals, or sometimes allowed to roam freely within the house [22]. Guinea pig owners use adobe, stacked bricks, stacked stones, chicken wire, cardboard boxes, and combinations of these materials to build their enclosures. In some households, people purchase guinea pigs from the markets to raise them over a few weeks or months. In other cases, households raise guinea pigs permanently, and the number of specimens at any given time is a function of the availability of pasture, its price, and family or local celebrations [24].

1.5. Animal reservoirs of *Trypanosoma cruzi* and vector hosts in the Andes

The importance of different domestic animals for the presence of vectors, the parasite, and transmission of *T. cruzi* has been studied in different countries [26-28], and different species of mammals have been described as potential reservoirs of *T. cruzi*. Gurtler et al. in 2009 [25] described the host-feeding preferences of *Triatoma infestans* in Argentina, reporting that *T. infestans* prefer to feed on dogs as compared to chicken and cats. They did not compare dogs and guinea pigs, since the latter is not commonly raised in Argentina. In addition, some species have been associated with increased numbers of vectors. In Peru, some studies have indicated the association between guinea pigs and an increased number of *T. cruzi*-infected vector in animal corrals [13,29,30].

The host-feeding preferences of *T. infestans* in the Peruvian Andes have not been evaluated, and both domestic dogs and guinea pigs should be considered. If host-feeding preferences are stronger for guinea pigs in the Peruvian Andes, it may lead to the aggregation of triatomine insects in and around guinea pig corrals. The differences in the life histories between different domestic species should be also considered. Guinea pigs usually live for a few months in rural communities in

Peru, while dogs live for 7 years in average. Also, guinea pigs are very stationary, meaning that usually they do not live their cages or corrals their whole lives. On the contrary, dogs roam around their houses, and can be exposed to infected triatomines beyond the boundaries of their housesholds. Therefore, domestic dogs are an important domestic species because they live for several years in proximity to humans, and if infected, may serve as a reservoir for *T. cruzi* during their lifespan. Guinea pigs might be also important species in the ecology of *Trypanosoma cruzi* and *Triatoma infestans* since there are several or many guinea pigs in the corrals. These groups of guinea pigs in corrals create subpopulations that might allow transmission of the parasite at a very small scale and their corrals offer good hiding and breeding places for the vectors.

1.6. Present studies about presence, abundance, and dispersion of *Triatoma infestans* and indicators of reemergence of *Trypanosoma cruzi*

The following manuscripts use data from community- and lab-based studies in Arequipa, Peru to explore three different aspects of the role of domestic animals in *T. infestans* ecology. The first study describes the association between different animal corral ecotopes (defined by each unique combination of animal species and construction materials per corral) and the presence and density of vectors and parasite-infected vectors. We find that ecotopes containing guinea pigs are strong predictors for the presence of both large colonies of *T. infestans* and for *T. cruzi*-

infected *T. infestans*. We discuss the implications of these findings for vector control.

The second study evaluated the sero-prevalence of *T. cruzi* infection in domestic animals, and found that seropositive dogs were located near large colonies of infected vectors. We also found sero-positivity in dogs increased with age, suggesting a risk of transmission after birth, consistent with vectorial transmission. The potential use of dogs as animal sentinels in areas of reemerging transmission of *T. cruzi*, is an option for improving vector control strategies.

Finally, the third study was conducted in our field entomology laboratory and used experimental data to understand the effect of population dynamics of domestic animal hosts on the presence and dispersion of triatomine vectors. We observed that triatomine insects disperse significantly even when the presence of hosts remains constant. After removal of hosts, the pattern of insect dispersal changes. The observed responses by triatomines to perturbed and unperturbed environments may be the result of adaptive survival strategies and have implications for vector surveillance and control.

In areas where residual insecticide has been applied to homes (the most common vector control measure for Chagas disease), transmission can reemerge if domestic animal reservoirs and insect colonies in animal corrals are not addressed. In conjunction, these three studies advance the understanding of the role that domestic animals, in particular dogs and guinea pigs, play in maintaining vector-borne transmission of *T. cruzi* and can inform improved vector control strategies.

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2. Factors associated with *Triatoma infestans* infestation, colonization and infection with *Trypanosoma cruzi* in areas with reemerging transmission in Arequipa, Peru

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2.1. Abstract

Background: Animal corrals are discrete geographical units that are built with specific materials and contain specific animal species. The materials comprise a habitat that can be used by triatomine vectors, and the animal species might interact with triatomine vectors (parasitism) to create a particular niche. The combination of habitat and niche produces an ecotope, and different ecotopes might facilitate or impede the infestation and reproduction of triatomine vectors. Also, the presence and dynamics of specific animal reservoirs might increase *T. cruzi*-infection in the vector population. The objective of this study was to determine the association between animal species and *T. cruzi*-infected vectors and ecotopes and vector infestation and colonization.

Methods: We conducted a cross-sectional entomological survey in four rural communities of Arequipa, Peru. We collected vectors, classified them based on developmental state and sex, and evaluated them for the presence of *T. cruzi*. We recorded the presence of domestic animals in the sites of capture, the materials used to build the animal enclosures, use of insecticide, and age of the corral. In houserooms, we recorded the same information and the number of persons sleeping in each room. With logistic regression models we evaluated the factors and ecotopes associated with the presence of vectors, nymph instars, and *Trypanosoma cruzi*-infected vectors. With zero-inflated binomial and Poisson regression models, we assessed the association between ecotopes and the count of vectors, the count of 5^{th} nymph instars, and the count of *T. cruzi*-infected vectors.

Results: Overall, empty corrals, rabbit corrals, and guinea pig corrals were the most infested (15% - 29%), colonized (12% - 25%) and infected (31% - 44%), and corrals made with stacked stones and bricks increased the number of vectors and nymph instars in almost any animal species. Compared to dogs, guinea pigs were strongly associated with the number of *T. cruzi*-infected vectors (mean ratio = 218.2; 95% CI: 54.1, 874.4). Within houses, the presence of humans increased the odds of infestation (OR = 4.6; 95% CI: 1.8, 11.8) and colonization (OR = 5.0; 95% CI: 1.8, 13.9), and also the number of humans increased the odds of infestation (OR = 1.7; 95% CI: 1.2, 2.4) and colonization (OR = 1.8; 95% CI: 1.3, 2.4).

Conclusions: Vector control programs in endemic areas will benefit from encouraging the use of wire mesh enclosures for all food animals, promoting raising animals other than guinea pigs, and including empty corrals in the vector surveillance activities.

Keywords: *Triatoma infestans*, *Trypanosoma cruzi*, ecotope, animal host, animal reservoir.

2.2. Introduction

American trypanosomiasis, or Chagas disease, is a vector-borne parasitic disease that affects 7 to 8 to million people in the Americas [1], of which approximately 192,000 are Peruvian [2,3]. The disease extends over most of the Americas region, and although recently it has become urbanized [4,5], the disease remains strongly associated with rural areas and its burden concentrated on underserved populations [6]. The vector of Chagas disease are insects of the subfamily triatominae and are typically nocturnal, taking blood meals from a wide range of animals, including mammals, birds and humans. The causative parasite, *Trypanosoma cruzi*, infects humans and other mammals [7], including dogs and livestock, which are common domestic animals in rural settings. The presence of domestic animals near households may make them important factors for the risk of triatomine infestation and *T. cruzi* transmission to humans.

Domestic animals can play a role in *T. cruzi* transmission as a source of blood meals for the triatomine vector and as a mammalian host for the *T. cruzi* parasite capable of infecting vectors. In addition, domestic animal corrals, pens or sleeping areas can harbor insect nests and colonies. The association between different domestic animal species and Chagas disease has been the subject of several studies from the United States to Argentina [8-13], and it seems that the importance of an animal species in vector infestation is context dependent. In some rural areas of Argentina dogs and chickens have been described as important hosts for triatomine vectors [14] and in other rural areas of the same country, goat corrals seem to play an important role in the nesting and persistence of triatomine populations [15]. In general, dogs have been described as important hosts for the vectors and reservoirs of the parasite of Chagas disease in many endemic countries, such as Mexico, Venezuela, Colombia, Peru, Brazil, and Argentina, [9,10,13,16-20]. In the Southern Andes, an autochthonous domesticated species may be an important reservoir of *T. cruzi*: the guinea pig.

Guinea pigs were domesticated in the Peruvian Andes about 7,000 years ago [21]. Since then, these small and resilient rodents have been raised as source of protein for Andean villagers. Domestication of guinea pigs later expanded south, reaching human communities in Bolivia where, around 4,500 years ago, populations of *Triatoma infestans,* a Chagas disease vector, which lived with and fed on wild guinea pigs, adapted to human dwellings and became a strictly domiciliary insect species, likely by following domesticated guinea pigs [22]. Many triatomine species can transmit *T. cruzi* [23], but in the department of Arequipa, Peru, *T. infestans* is the only insect vector present [19]. In this part of the Andes, the vector and the causative parasite of Chagas disease have probably coexisted with guinea pigs for more time than with any other domestic animal species, as many domestic animals were not introduced to the Americas until the 15th century.

In Peru, a positive association between guinea pigs and the presence of *T. infestans* in their corral has been reported since 1955 [24] and, in the same year, an association between *T. cruzi*-infected *T. infestans* and guinea pig corrals was also reported [25]. More recently Levy et al. [19] reported that guinea pig corrals had

2.4 times more *T. infestans* individuals compared to the corrals of other animal species. In addition, Levy et al. found that the presence of chickens is associated with fewer *T. infestans* individuals in corrals [19], possibly related to predation by chickens on vectors. Researchers in other countries have similarly reported fewer *T. cruzi*-infected vectors associated with the presence of chickens in experimental [26] and field settings [27], a finding associated with the refractory nature of birds to infection.

Domestic animals are usually enclosed in corrals, and in underserved and rural areas, animal corrals are often constructed with rustic materials. The materials used to build animal corrals and their role on the quantity of *T. infestans* and *T. cruzi* infections have also been studied. Levy et al. [19] found that animal corrals with wire mesh and stuccoed walls can be protective against *T. infestans*, whereas rustic materials, such as adobe and unmortared bricks are associated with larger number of *T. infestans*. On the contrary, Gorla et al. in Argentina [15] found no protective effect of improved goat corrals built with concrete and wire.

Vector control measures play a key role in the prevention of Chagas disease, and in the case of areas with *T. infestans*, insecticide residual application in households of areas endemic for Chagas disease is the main intervention employed [28]. In Arequipa, Peru, an insecticide residual spray campaign has been led by the Ministry of Health. Generally, insecticide is applied to walls inside houses, as well to walls and surfaces in the peridomiciliary area, including animal corrals. The insecticide used in this campaign (Deltamethrin 5%) has an immediate repellant effect on *T. infestans* and a long-acting killing effect on the insects [29]. Although reinfestation is probable, reemergence of *T. cruzi* transmission is a threat when mammalian parasite reservoirs persist in the area.

In Arequipa, Peru, the rural district of La Joya has been historically considered an endemic area of Chagas disease. This area had been sprayed with insecticide in 1996 to control triatomine insect infestation [30]. However, in 2008 the high level of reported *T. infestans* in this area indicated reinfestation and potential reemergence of transmission after 1996 that might have been facilitated by the presence of specific ecotopes in the area favorable to *T. infestans*.

An ecotope can be defined as the smallest ecologically distinct landscape. Ecotopes are created by the combination of environmental conditions (habitat) and the interactions of adaptively related species (niche) [31]. In the peridomiciliary area of houses in triatomine-infested regions, an animal corral with a specific set of animal species and discrete building materials can be considered an ecotope. Triatomine insects of different species show strong predilections for specific ecotopes, and this might influence evolution and behavior of the vector and *T. cruzi* [32].

Specific combinations of domestic animal species and corral materials may provide favorable ecotopes for *T. infestans*. The objective of our study was to determine the domiciliary and peri-domiciliary factors associated with the presence and abundance of *T. infestans* and *T. cruzi*-carrying *T. infestans*, in a setting of reinfestation and reemerging transmission. Special attention is placed on identifying those factors associated with the presence of nymphs, and indicator of colonization and suitability of the ecotope for *T. infestans*.

2.3. Methods

2.3.1. Ethical statement

The Institutional Review Board of Universidad Peruana Cayetano Heredia reviewed and approved all animal-handling protocols used for this study.

2.3.2. Study population and area

This study was conducted in four contiguous rural communities in the district of La Joya, with a population of approximately 2,500 persons in 677 households. The district of La Joya, located 30.7 km west (Euclidean distance) of the department capital city of Arequipa, sits at an altitude of 1617 m, has an average temperature of 18°C (range 10°C to 35°C), humidity ranging between 20% and 85%, and a marked rainy season. The residents of La Joya raise domestic animals for various uses. The most common species raised are guinea pigs, rabbits, dogs, cats, and poultry. It is a historically endemic area for Chagas disease and the district underwent residual insecticide application in September to November 2008 as part of the vector control program run by the Ministry of Health.

2.3.3. Study design

In collaboration with the Ministry of Health insecticide application program, we conduced a cross-sectional household study to collect entomological, parasitological and ecotope data in this rural settlement. In this context, an ecotope was defined as the smallest ecologically distinct landscape feature with shared construction materials and containing the same animal species; therefore, each animal corral was considered a unique ecotope. Corrals containing a combination of small mammals (rabbits or guinea pigs and poultry) were also considered unique ecotopes. Peridomiciliary ecotopes were the structures surrounding the house (e.g. animal corrals in the backyards or animal cages on the roof).

2.3.4. Entomological and parasitological data collection

We conducted a cross-sectional entomological survey in each house at the time of insecticide application by the Ministry of Health spraying campaign. In each house a field biologist, who accompanied the MoH insecticide sprayer, surveyed all the intra- and peri-domiciliary areas of the house, collecting *T. infestans* as they emerged in response to the insecticide. The search and collection was conducted over one hour/person/house. All bugs captured within the same room, animal corral, or other area of the house were placed in a jar and assigned a unique code corresponding to the room, corral or area of capture. The captured triatomine insects were sent to our field lab in the city of Arequipa where their sex and developmental stage were determined. We diagnosed the presence of *T. cruzi* in all insects, except first instars, by direct microscopy observation of gut contents. Direct microscopy has shown sensitivity of 90.5% and specificity of 80.4% in *T.*

cruzi infection diagnosed in triatomines from the field [33]. The entomological information was linked to the data collected on the rooms and corrals where insects were captured.

2.3.5. Ecotope data

The characteristics of each room and corral in all surveyed houses were recorded. We collected information by direct observation on type of enclosure (animal corral or house room), purpose of room (e.g. storage, bedroom), the materials used to build rooms and corrals, and animal species kept in that environment. We also recorded if humans slept in any of the rooms. Domiciliary ecotopes were classified based on their function (e.g. kitchen, bedroom), and peri-domiciliary ecotopes were classified based on the animal species they contained (e.g. guinea pig corral).

2.3.6. Statistical analysis

We estimated the prevalence of *T. infestans* and *T. cruzi* in the houses, rooms and corrals, and the index of colonization (the proportion of infested areas where nymphs were found). We dichotomized the presence of triatomine vectors to define infested corrals and houserooms, we dichotomized the presence of nymph instars to define colonized corrals and houserooms, and finally, we also dichotomized the presence of *T. cruzi*-infected triatomine vectors to define infected corrals and houserooms. Based on these dichotomous categories we reported the prevalence of infested, colonized and infected corrals by animal species. We focused on the most prevalent animal corrals and houserooms for subsequent analyses.

Animal species and corral materials

We used logistic regression models to evaluate the animal species and corral materials associated with the presence of triatomine insects (infestation), presence of nymph instars (colonization) and *T. cruzi*-carrying triatomine insects (infection). We also evaluated the count of triatomines, nymph instars, and *T. cruzi*-carrying triatomines. First nymph instars are very small compared to adults. The size of nymph instars increases progressively and the size of a fifth nymph instar is not very different from the size of adults. To prevent differential reporting of triatomines biased by developmental stage (detection probability associated with size), we used only data of adults and fifth instar as surrogates of insect population to evaluate the density of vectors per capture site. To model the count of total vectors and the count of 5^{th} nymph instars in each site of capture we used a zero-inflated negative binomial regression without regressors for the zero component of the model. The count of total *T. cruzi*-infected vectors was modeled with zero-inflated Poisson regression. In all regression analyses, for factors associated with infestation, colonization, and infection we considered corrals of dogs, guinea pigs, poultry, rabbits, poultry and small mammals together, empty corrals, material of construction, time of construction, and use of insecticide within the last 6 months. Dogs have been associated with vector infestation and *T. cruzi*-infected vectors in different countries [9,20,27,34]. For reference group we used dogs when analyzing the effect of animal species, and doghouses made of mortared bricks or stones when analyzing the effect of ecotopes.

Ecotopes

With a heat map we displayed the distribution of corral materials for the most prevalent animal species to determine the most prevalent ecotopes in the study area. We chose those corral materials that were present in at least 10% of each animal species corrals to analyze the association between ecotope and infestation and colonization. The association between ecotopes and infestation and colonization was assessed with logistic regression models, and the association between ecotopes and the count of *T. cruzi*-infected triatomines was assessed with zero-inflated Poisson regression models.

Rooms of the human dwelling

We modeled infestation, colonization and infection of vectors within household rooms as a function of the presence of human sleeping spaces, number of humans sleeping in the room, and presence of animals in the bedroom. For this analysis, we used logistic regression models adjusted for the bedroom construction materials.

All statistical analyses were performed with R and estimates were evaluated with an alpha level of 0.05.

2.4. Results

Of 677 households in the study area, 612 were surveyed (participation rate 90.4%); of these 612 houses, triatomine insects were found and collected from 168 (27.5%) houses, and *Trypanosoma cruzi*–carrying triatomine insects were collected from 46 of the 168 infested houses (27.4%). Of 3,753 rooms in human dwellings surveyed, 187 (5%) were infested with vectors and 18 (0.5%) contained *T. cruzi*–carrying triatomine insects. Of 1,762 animal enclosures, 204 (11.6%) were infested with vectors and 60 (3.4%) had insects carrying the parasite. One hundred thirty one rooms and 177 corrals had fifth nymph instars; therefore, the colonization index (number of sites with triatomine nymphs/number of sites with triatomines) for rooms was 70.1% and for animal enclosures was 86.8%.

We found strong interspecific variability in levels of infestation, colonization, and infection. A total of 9,557 triatomine insects were captured; 2,070 in human dwellings and 7,487 in peridomestic areas. Most *T. cruzi*-carrying triatomine insects were captured in peridomestic areas (96.7%). At the time of the survey, 497 (81.2%) households in La Joya kept domestic animals, but only 47 households (7.7%) allowed some companion animals to sleep inside at night. The rest of the animals were kept in the peridomiciliary area.

Table 1.1 and Figure 1.1 show the levels of infestation and colonization across corrals housing different animal species. Surprisingly, empty corrals had the highest levels of infestation (29%) and colonization (25%), followed by rabbit corrals (15% and 13%, respectively), and guinea pig corrals (13% and 12%, respectively). Corrals of other animal species had infestation and colonization levels below 10%. Among infested corrals, the presence of *T. cruzi*-infected triatomines showed wide variability across animal species corrals (Figure 1.2). We found *T. cruzi* infected triatomines in 44% of infested guinea pig corrals, 31% in infested empty corrals, 22% in infested poultry corrals, and 18% in infested doghouses. Corrals of other animal species had lower infection levels or were not very prevalent in the study area (Figure 1.2).

The presence of any vector and nymph instars was dependent on animal species, but not on corral materials, nor use of insecticide in the last six months or time since construction of the corral (Table 1.2). Compared to doghouses, guinea pigs corrals had odds of infestation 3.5 times higher (95% CI: 1.8, 7.1) and odds of colonization 3.7 times higher (95% CI: 1.8, 7.6). Rabbit corrals had odds of infestation 4.3 times higher (95% CI: 1.6, 11.2) and odds of colonization 4 times higher (95% CI: 1.6, 10.4), and empty corrals had odds of infestation 10 times higher (95% CI: 5.3, 19.1) and odds of colonization 7.6 times higher (95% CI: 3.9, 14.9) when compared with doghouses. Despite these associations, the presence of *T. cruzi*-infected vector was not statistically associated with animal species (Table 1.2).

The dichotomous variables of infestation, colonization, and infection do not represent the insect density associated with specific animal corrals. Figure 1.4 shows that the majority of insects captured in the peridomiciliary ecotopes were found in guinea pig corrals, followed by empty corrals and poultry corrals.

Captured nymph instars were fewer and followed a similar distribution across animal species. Lastly, the *T. cruzi*-infected vectors were found almost entirely in guinea pig corrals. When we modeled the number of captured vectors (Table 1.3) we found that, compared to the average number of vectors captured in doghouses, the average number of vectors captured in guinea pig corrals was 25 times higher (95% CI: 10, 67), in poultry corrals was 4.1 times higher (95% CI: 1.8, 9.5), and in empty corrals was 8.9 times higher (95% CI: 3.6, 22.0). Compared to doghouses, the average number of fifth nymph instars captured in guinea pig corrals was 127 times higher (95% CI: 34, 471), in poultry corrals was 23 times higher (95% CI: 6.8, 77.6), and in empty corrals was 21.4 times higher (95% CI: 6.1, 75.8). The only animal species that showed a statistically significant difference of *T. cruzi*-infected vectors when compared to dogs was guinea pigs (Table 1.3) (Mean Ratio=218; 95% CI: 54, 874), although corrals without any animals inside had an average number of infected vectors 6.5 higher than those from doghouses (95% CI: 1.6, 26.3).

To evaluate the peridomiciliary ecotopes and their association with the vector we assessed the prevalence of corral materials by animal species. Some materials such as wood, stucco, adobe, and corrugated material were uncommon in most animal species corrals (Figure 1.3). According to Table 1.4, compared to doghouses made of mortared bricks or stones, empty corrals built with other materials had odds of infestation 15.9 times higher (95% CI: 3.4, 74.2) and odds of colonization 11.3 times higher (95% CI: 2.4, 54.1); empty corrals built with stacked stones or bricks had odds of infestation 42 times higher (95% CI: 10, 181) and odds of colonization 31 times higher (95% CI: 7, 133). Also, compared to doghouses made of mortared bricks or stones, guinea pig corrals with mortared stones or bricks had odds of colonization 1.3 times higher (95% CI: 3.9, 14.9), and guinea pig corrals with stacked stones or bricks had odds of infestation 8.5 times higher (95% CI: 1.8, 39.4) and odds of colonization 7.7 times higher (95% CI: 1.7, 35.9).

The vector density or count of vectors per corral varied greatly by ecotope. In Table 1.4 we see that, compared to the average number of vectors captured in doghouses made of mortared stones or bricks, the average number of vectors are higher in ecotopes that contain the following species: guinea pigs, rabbits, poultry, and mammals and poultry together. In some cases, the increase in the number of vectors is accentuated by the presence of specific materials such as stacked stones or bricks. Wire mesh showed different effects across ecotopes; in rabbit corrals and corrals with mammals and poultry together it was protective while in guinea pig or poultry corrals wire mesh increased the average number of vectors.

Within households, the presence of sleeping humans in a room increased the odds of infestation by 4.6 times (95% CI: 1.8, 11.8) and the odds of colonization by 5 times (95% CI: 1.8, 13.9) (Table 1.5). With each extra person sleeping in the room the odds of infestation increased by 1.7 times (95% CI: 1.2, 2.4) and the odds of colonization increased by 1.8 times (95% CI: 1.3, 2.4) (Table 1.5). The odds of infected vectors in houserooms were not associated with the presence of humans or the number of persons in the room. The number of houses that allowed domestic animals to sleep inside was small, and no association was found between the presence of animals in human bedrooms and triatomine infestation, colonization or infection.

2.5. Discussion

In this work conducted in rural communities, the most infested ecotopes were corrals built with stacked stones or bricks and containing guinea pigs or no animals. Levy et al. [19], in a study that took place in a periurban community also located in the department of Arequipa, obtained similar results regarding guinea pigs and stacked stone corrals. The four communities that comprised our study area showed high levels of infestation and colonization in the peridomiciliary area, while infestation and colonization were lower in the domiciliary rooms. Almost all *T. cruzi*-carrying vectors were found in peridomiciliary animal corrals. These findings suggest a process of reinfestation that occurred after insecticide spraying in 1996 and started and grew in the peridomiciliary area. This reinfestation process was followed by reemergence of transmission, also in the peridomiciliary area. Lastly and more recently, *T. cruzi*-carrying vectors are present in the domiciliary rooms, probably by recent colonization of rooms (breeding within rooms) or by migrating insect individuals from peridomiciliary corrals into houserooms.

The influence of ecotope characteristics on phenotypic expressions, such as color and size of the triatomine insects, has been suggested [35], but we did not perform any phenotypic characterization of the captured vectors. These ecotope-dependent phenotypic changes might influence reproductivity parameters as well, and might be related to the higher number of insects and higher indices of colonization of some ecotopes. A component of ecotopes is the animal species present (vector hosts). The effect of blood meal source on the variation in the levels of infestation should not be underestimated. Nattero et al. [36] found experimentally that *T. infestans* fed with guinea pig blood showed higher reproductive parameters compared to those fed with pigeon blood.

Gaunt and Miles [32] suggested that *Triatoma* species are associated with rocky habitats or rodent burrows. In our study area there was an important presence of corrals made of mortared or stacked stones and bricks. The high count of vectors and nymph instars in these rocky habitats occupied by small mammals (guinea pigs and rabbits) reinforces the hypothesis of *T. infestans* adapted to these ecotopes. In Brazil, a study of peridomiciliary ecotopes reported high numbers of other *Triatoma* species, *T. brasiliensis* and *T. pseudomaculata,* found in straw roofs, roofing tiles, and brick piles and mainly associated with goat/sheep corrals [37]. They did not report the presence of domestic rodents in their study area, so the difference is likely related to the availability of animal hosts.

The reservoir capacity represents the potential ability of a host to sustain transmission of a pathogen and it is determined by infectivity timing with vector activity, abundance of the reservoir, and contribution to infection in the vector. The high prevalence of *T. cruzi* in insects captured in guinea pig corrals and the abundance of guinea pig corrals in the study area suggest a very high reservoir capacity. Reservoir competence is the ability of a host to make a pathogen available to a vector and it is related to reservoir capacity. Our team has shown under laboratory conditions that reservoir competence is high in guinea pigs with more than 90% of vectors infected when fed on guinea pigs and most infected guinea pigs being infectious for months (data not published). In corrals with only guinea pigs all vector activities are likely focused on feeding on guinea pigs, increasing the probability of infection and the overall vector population. The presence of other host species with lower reservoir competence might reduce the chances of vector becoming infected.

The reduced prevalence of *T. cruzi* in insects captured in corrals containing small mammals cohabitant with poultry suggests that poultry has a high capacity of reducing the overall reservoir competence in a corral, a phenomenon that has been described in other infectious diseases as the “dilution effect” [38]. Birds are refractory to *T. cruzi* infection [39], and they can clear infection as fast as 7 minutes after the parasite reaches bloodstream [40]. Experimentally, it has been shown that the presence of chickens around infected guinea pigs reduces *T. cruzi* infection in triatomine insects [26]. For that experiment the researchers did not place chicken and guinea pigs together in the same cage, but rather in individual cages close to each other. Based on our observations it is possible that this makes a difference in the effect of birds on infection level in the vectors. In the mixed corrals we surveyed in our study, insects have to choose to feed on guinea pigs or poultry reducing the net transmission of the parasite. Moreover, positive insects that feed on poultry might clear the infection and become negative since birds blood contain immune complement that can lyse *T. cruzi*. Figure 1.5 depicts this potential infection-clearing mechanism and some of the key elements about that transmission system.

We did not find any *T. cruzi*-infected vector in the infested corrals containing mammals and poultry. There were only eight of those corrals, but the observation deserves an elaboration of its potential causes. Zooprophylaxis has been defined by the World Health Organization as the diversion of pathogen carrying insects from humans to animals [41]. This dilution effect states that the increase of host-species richness decreases the overall prevalence of the *Borrelia burdorgferi* and decrease risk of Lyme disease for humans [42]. In these rural areas where small-scale economies promote productivity systems based on small animal species, an increase of mixed (poultry-mammal) corrals could help to decrease the prevalence of *T. cruzi* in vectors. This type of production system has proven to be cost-effective in rural areas of the Andes where guinea pigs in combination with other small species are raised [43], and is already practice by many dwellers in the Andes. Therefore, its promotion and implementation should not face cultural or economic resistance.

Importantly, empty corrals were the peridomiciliary enclosures most infested with and colonized by vectors, and highly infected with *T. cruzi*. Similarly, in Parana, Brazil, uninhabited houses were the most infested structures by different triatomine species, and they were followed by chicken coops [44]. In anecdotal conversations with dwellers in our study area we learned that the turnover of domestic animals is high and, in some cases, it is seasonal. During the dry season the price of pasture increases, so some small farmers decide to slaughter their farm animals and wait until the dry season ends to obtain and raise new farm animals. The high and seasonal turnover of domestic animals might explain the high presence of vectors in empty corrals. The high presence of *T. cruzi*-carrying vectors in empty and guinea pig corrals suggests that guinea pigs occupied those empty corrals before we conducted the study. This indicates that after insecticide spraying surveillance should focus heavily on empty and guinea pig corrals, built with stacked or mortared stones and bricks.

We observed a positive association between infestation and colonization in houserooms and the presence and number of persons sleeping in the room. In Argentina, the presence of dogs and cats within houses was associated with an increased number of vectors and infected vectors within the houses [10]. In our study we did not observe such association. There was a small number of domestic animals that slept within the houses in this study area. That might have reduced the migration of vectors from the peridomicilary area into the domiciliary area and posterior colonization.

As with any observational study, our findings should be observed with caution. What we observed was a snapshot of a dynamic system in which vector populations, domestic animal presence, and transmission of the parasite might be changing. We did not collect information on which domestic animal species habited the empty animal corral before our study was conducted. The presence of triatomine predators such as geckos, spiders, and scorpions could also shape the vector population in the area, but we did not collect information on those predators. There is also the possibility of threats to accuracy during fieldwork and lab work. During the entomological surveys in the field, some animal enclosures could have been defined as non-infested because fieldworkers did not detect the presence of triatomines. This is known as non-detection bias in ecology and, in our case, might be associated with the quantity of vectors in an area and the developmental stage of the vectors. Finally, the parasitological analysis in the lab to detect *T. cruzi* was done by direct microscopy. Compared to PCR analysis this method has 91% sensitivity and 80% specificity; therefore, some false positives and false negative could be expected.

2.6. Conclusions

The high colonization indices suggest that the infestation by *Triatoma infestans* was well established and must have begun many years ago, but not further than 1996 when the study area was first sprayed with residual insecticide [30]. It is clear that peridomiciliary animals, guinea pigs in particular, and corrals not made of wire mesh are important risk factors for the presence of triatomine insects and *T.cruzi*-carrying triatomine insects. However, keeping small animals in the surroundings of the house is an important source of protein for rural families, and raising guinea pigs for human consumption is a common and long-standing custom in Arequipa. Birds cannot transmit the parasite, but in out study raising poultry was also associated with abundance of *T. infestans*. For this reason, vector control programs in endemic areas will benefit from encouraging the use of wire mesh enclosures for all food animals, promoting raising animals other than guinea pigs, and including empty corrals in the vector surveillance activities.

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Table 1.1. Infestation, colonization and infection levels by ecotopes

Ecotopes	N	% Infested	% Colonized	% Infected
Peri-domiciliary	1813	11.4	9.8	28.5
Poultry	486	6.6	5.6	21.9
Dogs	275	4.0	3.3	18.2
Guinea pig	265	12.8	12.1	44.1
Small mammals and Poultry	113	7.1	6.2	0.0
Cat	85	1.2	1.2	0.0
Sheep	66	9.1	6.1	33.3
Rabbit	53	15.1	13.2	12.5
Pig	43	4.7	4.7	0.0
Cow	33	3.0	3.0	0.0
Other species	41	0.0	0.0	NA
Empty	353	29.5	24.9	30.8
Domiciliary	3596	5.4	3.8	10.8
Bedroom	1196	9.1	6.5	7.3
Storage room	585	4.3	3.1	16.0
Bathroom	536	0.6	0.2	33.3
Kitchen	516	2.7	1.2	21.4
Living room	125	1.6	0.8	0.0
Dinning room	90	1.1	1.1	0.0
Other	548	7.5	5.8	12.2
Total	**5409**	**7.4**	**5.8**	**19.9**

Infested: presence of *T. infestans*, Colonized: presence of any nymphal stage of *T. infestans*, Infected: presence of *T. cruzi* in infested corrals.

Table 1.2. Factors associated with presence of vectors estimated with logistic regression models

Associated factor	Infestation: Presence of any stage of *T. infestans* OR (95% CI)	p	Colonization: Presence of nymph instar OR (95% CI)	p	Infection: Presence of *T. cruzi*-infected vector OR (95% CI)	p
Animal species						
Dog	Reference	--	Reference	--	Reference	
Guinea pig	3.5 (1.8, 7.1)	<0.01	3.7 (1.8, 7.6)	<0.01	3.6 (0.7, 19.0)	0.13
Rabbit	4.3 (1.6, 11.2)	<0.01	4.0 (1.6, 10.4)	<0.01	0.6 (0.1, 8.6)	0.74
Mammal and Poultry	1.8 (0.7, 4.7)	0.21	1.9 (0.7, 5.0)	0.19	0.0 (0.0, 10.0)	0.99
Poultry	1.7 (0.8, 3.4)	0.14	1.7 (0.8, 3.6)	0.16	1.3 (0.2, 7.2)	0.80
Empty	10.0 (5.3, 19.1)	<0.01	7.6 (3.9, 14.9)	<0.01	2.0 (0.4, 9.8)	0.39
Construction materials						
Adobe	Reference	0.03	Reference	--		
Stacked brick or stone	1.3 (0.3, 5.7)	0.77	1.0 (0.3, 3.6)	1.00		
Corrugated material	0.6 (0.1, 3.5)	0.61	0.7 (0.2, 2.9)	0.60		
Mortared brick or stone	0.5 (0.1, 2.6)	0.44	0.5 (0.1, 1.8)	0.26		
Stucco	0.2 (0.0, 2.6)	0.22	0.0 (0.0, 0.0)	0.97		
Wire mesh only	0.3 (0.1, 1.5)	0.16	0.4 (0.1, 1.4)	0.13		
Wood	0.4 (0.1, 2.1)	0.28	0.4 (0.1, 1.5)	0.16		
Other material or combination	0.5 (0.1, 2.7)	0.45	0.5 (0.1, 1.9)	0.30		
Insecticide applied within last 6 months	1.0 (1.0, 1.0)	0.49	0.9 (0.6, 1.4)	0.54		
Time since construction of corral in years	1.0 (1.0, 1.0)	0.24	1.0 (0.9, 1.0)	0.31		

Table 1.3. Factors associated with density of vectors estimated with zero-inflated negative binomial regression and zero-inflated Poisson regression models

Associated factor	All *T. infestans* Mean ratio (95% CI)	p	5^{th} nymph instar Mean ratio (95% CI)	p	Infected *T. infestans* Mean ratio (95% CI)	p
Animal Species						
Dog	Reference	--	Reference	--	Reference	
Guinea pig	25.4 (9.7, 66.5)	<0.01	127.3 (34.4, 471.3)	<0.01	218.2 (54.1, 874.4)	<0.01
Rabbit	2.0 (0.4, 10.9)	0.41	2.6 (0.3, 25.2)	0.41	2.1 (0.3, 12.3)	0.43
Mammal and Poultry	1.3 (0.4, 4.5)	0.72	2.8 (0.5, 16.0)	0.24	0.0 (0.0, 1.6)	0.94
Poultry	4.1 (1.8, 9.5)	0.00	23.0 (6.8, 77.6)	<0.01	0.8 (0.2, 2.7)	0.75
Empty	8.9 (3.6, 22.0)	<0.01	21.4 (6.1, 75.8)	<0.01	6.5 (1.6, 26.3)	<0.01
Construction materials						
Adobe	Reference	--				
Stacked brick or stone	1.3 (0.1, 36.7)	0.88	NE		3.3 (1.1, 10.4)	0.04
Corrugated material	2.2 (0.1, 84.6)	0.66	NE		1.9 (0.6, 6.4)	0.30
Mortared brick or stone	0.3 (0.0, 7.7)	0.43	NE		0.3 (0.1, 0.9)	0.04
Stucco	0.0 (0.00, 0.8)	0.04	NE		0.0 (0.0, 7.6)	0.95
Wire mesh only	0.8 (0.0, 23.1)	0.89	NE		18.8 (6.0, 58.4)	<0.01
Wood	0.2 (0.0, 5.6)	0.32	NE		0.53 (0.1, 2.0)	0.35
Other material or combination	0.3 (0.0, 8.6)	0.46	NE			
Insecticide applied within last 6 months	1.0 (0.4, 2.6)	0.99	1.1 (0.4, 3.2)	0.89	1.9 (1.7, 2.1)	<0.01
Time since construction of corral in years	1.0 (0.9, 1.1)	0.71	1.0 (0.9, 1.2)	0.39	1.0 (0.9, 1.1)	0.12

NE: Not estimable

Table 1.4. Association between ecotopes and presence of vectors and parasite estimated with logistic and zero-inflated Poisson regression models

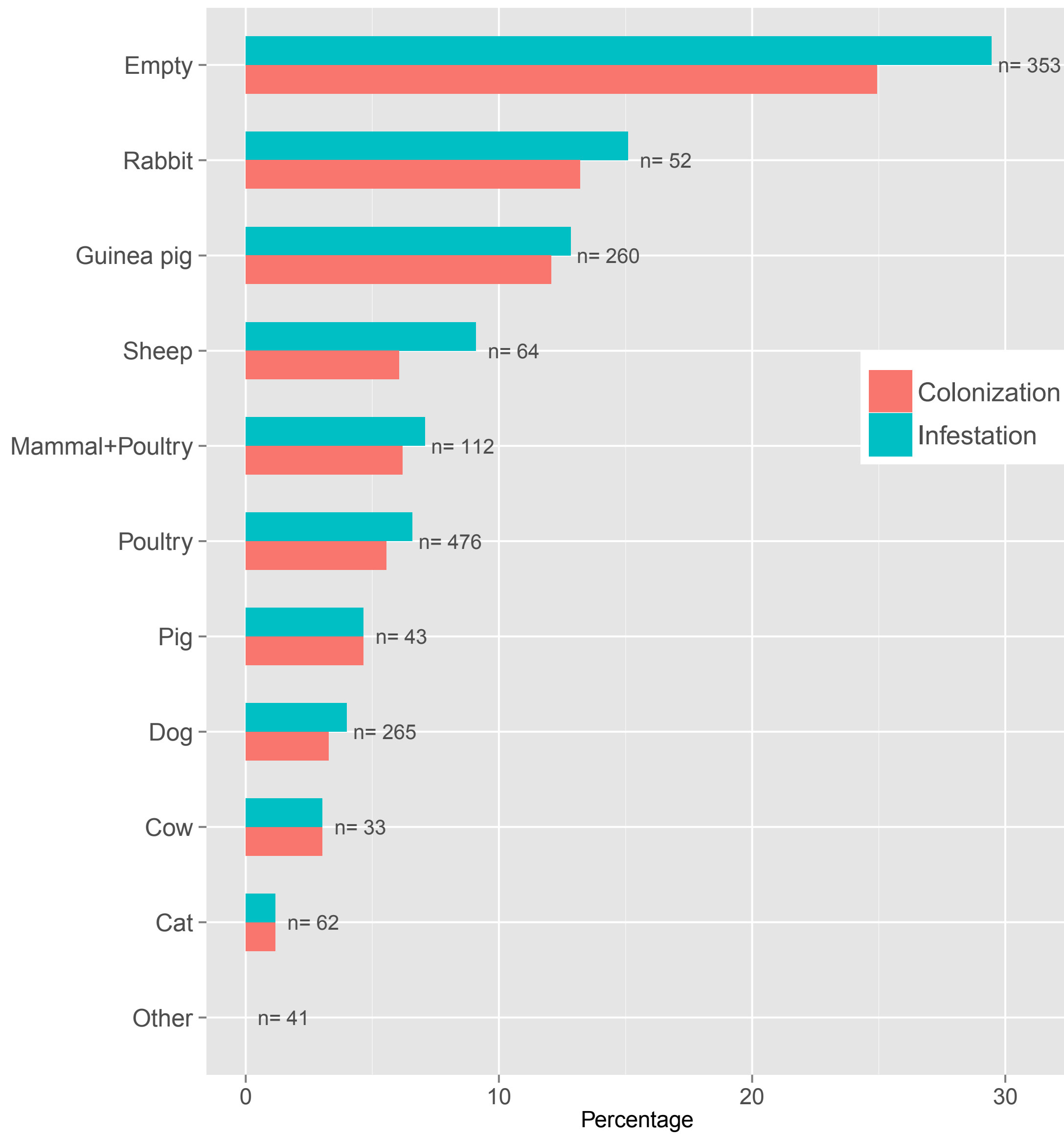
	Infestation: Any *T. infestans*		Colonization: Nymph instars		Insect Density All *T. infestans*	
Ecotope	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**	**Mean ratio (95% CI)**	**p**
Dog and mortared stone/brick	Reference	--	Reference	--	Reference	--
Dog and stacked stone/brick	2.6 (0.5, 12.7)	0.23	2.3 (0.5, 11.2)	0.32	9.7 (4.7, 19.8)	<0.01
Empty and other materials	15.9 (3.4, 74.2)	<0.01	11.3 (2.4, 54.1)	<0.01	34.0 (16.7, 69.1)	<0.01
Empty and stacked stone/brick	42.3 (9.9, 180.8)	<0.01	31.3 (7.3, 133.4)	<0.01	86.7 (43.3, 173.8)	<0.01
Empty and wire mesh	1.7 (0.3, 9.0)	0.53	1.7 (0.3, 9.0)	0.53	1.8 (0.8, 4.0)	0.18
Guinea pig and mortared stone/brick	1.3 (0.1, 14.5)	0.85	1.3 (3.9, 14.9)	<0.01	60.0 (29.6, 121.7)	<0.01
Guinea pig and stacked stone/brick	8.5 (1.8, 39.3)	<0.01	7.7 (1.7, 35.9)	<0.01	151.8 (75.8, 303.4)	<0.01
Guinea pig and wire mesh	4.8 (1.1, 21.6)	0.04	4.8 (1.1, 21.6)	0.04	104.8 (52.3, 209.9)	<0.01
Mammal and poultry and mortared stone/brick	3.0 (0.4, 22.2)	0.29	1.4 (0.1, 16.3)	0.78	1.4 (0.42, 4.65)	0.58
Mammal and poultry and stacked stone/brick	4.3 (0.8, 22.4)	0.08	4.3 (0.8, 22.4)	0.08	10.7 (5.1, 22.4)	<0.01
Mammal and poultry and wire mesh	0.0 (0.0, >10)	0.99	0.0 (0.0, >10)	0.98	0.0 (0.0, >10)	0.98
Poultry and mortared stone/brick	3.5 (0.7, 18.1)	0.13	2.9 (0.5, 15.4)	0.22	1.6 (0.7, 4.0)	0.28
Poultry and stacked stone/brick	4.2 (0.9, 19.1)	0.07	2.8 (0.6, 13.3)	0.20	52.6 (26.2, 105.7)	<0.01
Poultry and wire mesh	1.8 (0.4, 8.4)	0.47	1.8 (0.4, 8.4)	0.47	9.0 (4.4, 18.2)	<0.01
Rabbit and other material	13.6 (1.6, 117.9)	0.02	13.6 (1.6, 117.9)	0.02	36.3 (16.6, 79.3)	<0.01
Rabbit and wire mesh	0.0 (0.0, >10)	0.98	0.0 (0.0, >10)	0.98	0.0 (0.0, >10)	0.93

Table 1.5. Factors associated with presence of *T. infestans* in bedrooms estimated with logistic regression models

	Infestation		**Colonization**		**Infected *T. infestans***	
	OR* (95% CI)	**p**	**OR* (95% CI)**	**p**	**OR* (95% CI)**	**p**
Presence of humans	4.6 (1.8, 11.8)	<0.01	5.0 (1.8, 13.9)	<0.01	1.1 (0.2, 5.7)	0.88
Number of humans	1.7 (1.2, 2.4)	<0.01	1.8 (1.3, 2.4)	<0.01	1.2 (0.7, 1.9)	0.53

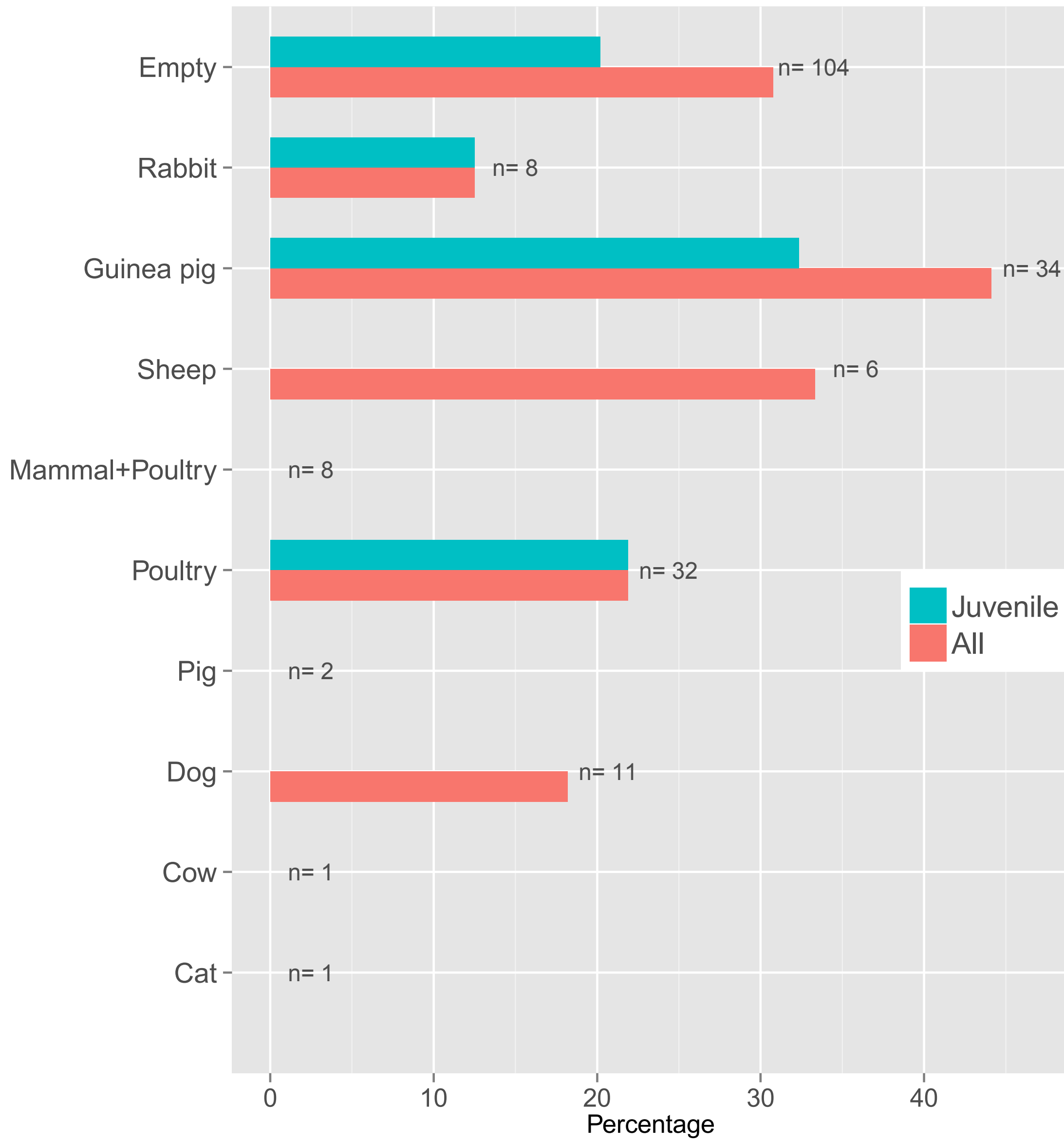
*Adjusted for the materials used to build the room

Figure 1.1. Presence of *Triatoma infestans* in animal corrals



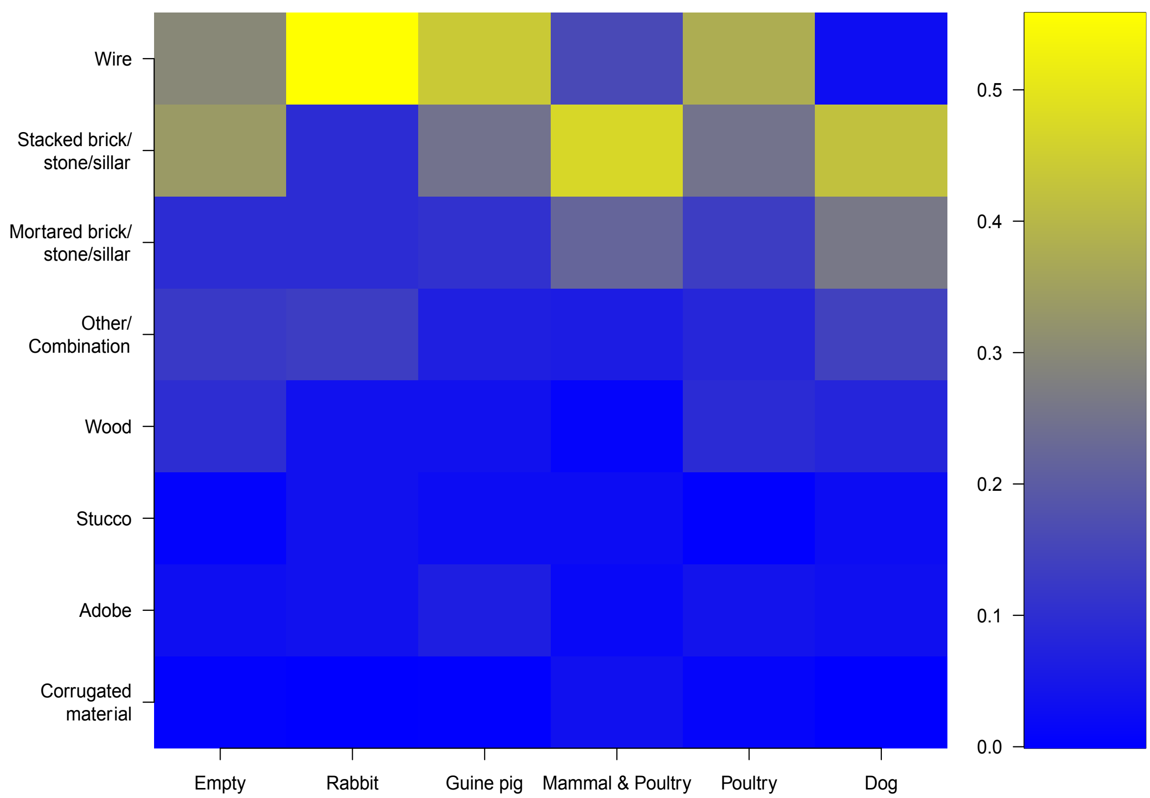
Infestation refers to the presence of any *T. infestans* individual and colonization refers to the presence of any nymph instar of *T. infestans*. The "n" is the number of surveyed corrals of that animal species.

Figure 1.2. Presence of *Trypanosoma cruzi*-infected *T. infestans* in animal corrals



Infestation refers to the presence of *T. cruzi* in any *T. infestans* individual and colonization refers to the presence of *T. cruzi* in juvenile *T. infestans* (any nymph instar). The "n" is the number of infested corrals of that animal species.

Figure 1.3. Proportion of different ecotopes based on animal species and corral materials



Cell color represents the proportion of corrals built with a specific material among all the corrals of a specific animal species.

Figure 1.4. Number of total triatomines captured, total nymph instars captured and total *T. cruzi*-infected captured by animal species corral

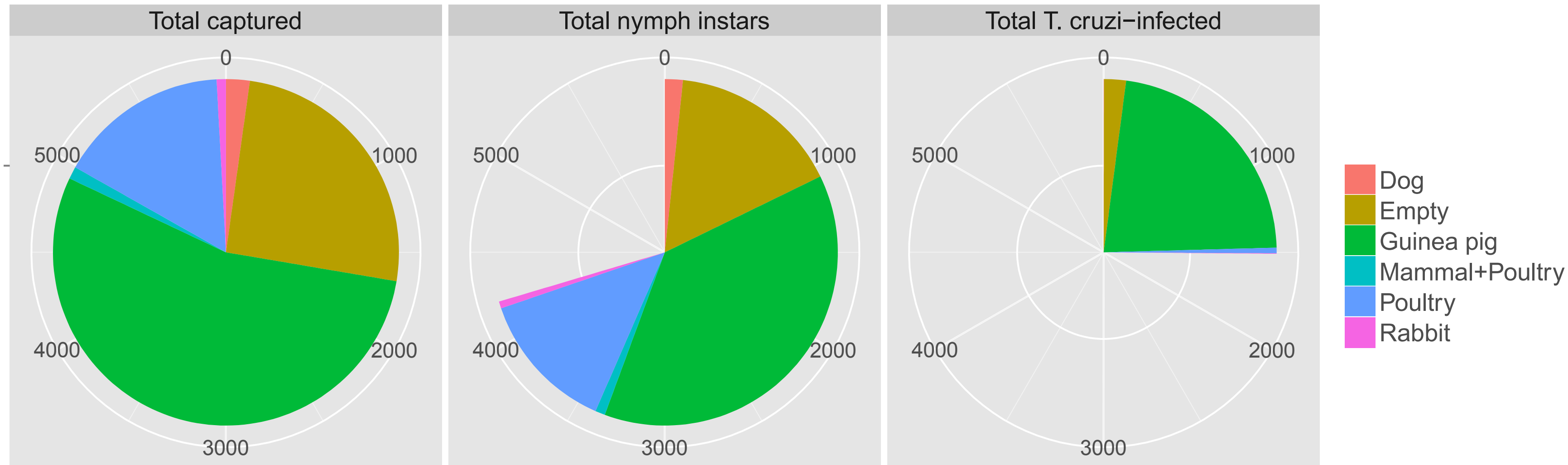
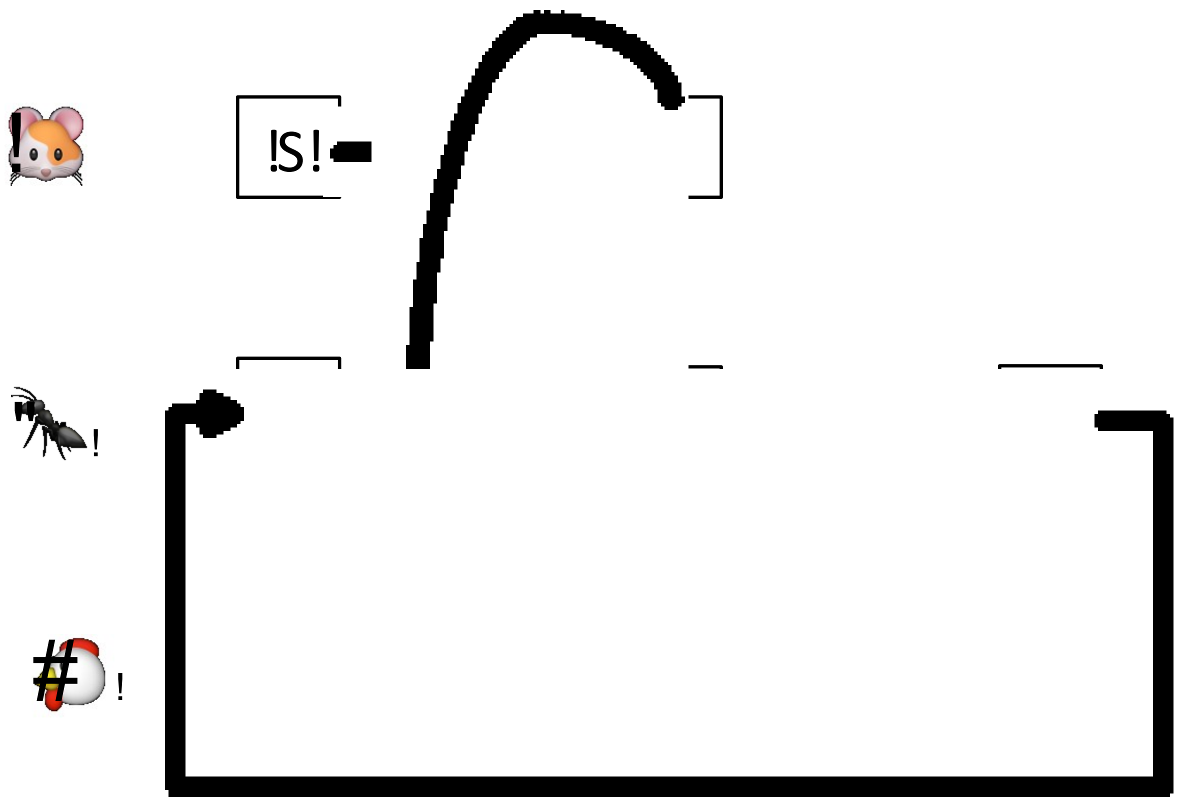


Figure 1.5. Model of poultry, guinea pigs, triatomine vectors and *Trypanosoma cruzi* transmission.



S: susceptible individuals in each animals species; I: *T. cruzi*-infected individuals in each animals species; R: individuals resistant to *T. cruzi* infection. Solid lines denote change of states and dotted lines denote interaction between species that lead to changes in state.

3. Dog seroprevalence of *Trypanosoma cruzi:* Potential animal sentinels of reemerging transmission in Arequipa, Peru

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3.1. Abstract

Background: Chagas disease, a vector-borne disease transmitted by triatomine bugs and caused by the parasite *Trypanosoma cruzi,* affects millions of people in the Americas. In Arequipa, Peru, indoor residual insecticide spraying campaigns are routinely conducted to eliminate *Triatoma infestans*, the only triatomine vector in this area. After insecticide spraying, there is risk of vector return and reinitiation of parasite transmission. Dogs are important reservoirs of *T. cruzi* and may play a role in reinitiating transmission in previously sprayed areas. Dogs may also serve as indicators of re-emergent transmission in an area.

Methods: We conducted a cross-sectional serological screening to detect *T. cruzi* antibodies in dogs, in conjunction with an entomological vector collection survey at the household level, in a disease endemic area that had been treated with insecticide 12 years prior. Spatial clustering of infected animals and vectors was determined using K-function difference, and the odds of being seropositive for dogs proximate to infected colonies was estimated with multivariate logistic regression.

Results: There were 106 triatomine-infested houses (41.1%), and 45 houses infested with *T. cruzi*-infected triatomine insects (17.4%). Canine seroprevalence in the area was 12.3% (n=154); all seropositive dogs were 9 months old or older. We observed clustering of vectors carrying the parasite, but no clustering of seropositive dogs. The adjusted odds ratio between seropositivity to *T. cruzi* and proximity to an infected triatomine (≤50m) was 5.67 (95% CI: 1.12 – 28.74;

p=0.036).

Conclusions: Targeted control of re-emerging disease can be achieved by improved understanding of *T. cruzi* in canine populations. Our results suggest that dogs may be useful sentinels to detect re-initiation of transmission following insecticide treatment. Integration of canine *T. cruzi* blood sampling into existing interventions for zoonotic disease control (e.g. rabies vaccination programs) can be an effective method of increasing surveillance and improving understanding of disease distribution.

Keywords: Dog, ELISA, *Triatoma infestans*, *Trypanosoma cruzi*, Sentinel surveillance, Spatial analysis.

3.2. Introduction

American trypanosomiasis, or Chagas disease, is a parasitic disease that affects 7 to 8 million people in The Americas [1], of which approximately 192,000 are Peruvian [2]. Most people infected with *Trypanosoma cruzi*, the parasite that causes Chagas disease, have mild or no symptoms during the acute phase of the disease (shortly after infection), and pass into the chronic phase of infection without being detected [3,4]. In the chronic phase, most infected individuals show no signs or symptoms and are considered to have the indeterminate form of the disease. However, it is estimated that 20 to 30% of those infected will develop cardiac or digestive forms of Chagas disease, usually decades after infection [3,4]. Clinical disease cannot be reversed and is often fatal [3-5]. Due to these characteristics, preventing new human cases is imperative to control Chagas as a public health problem.

The *T. cruzi* parasite is transmitted by triatomine insects that feed on humans and other mammals; reducing vector populations by insecticide spraying is one of the most effective approaches [6] to reducing parasite transmission and preventing human cases of Chagas disease. However, after an area is sprayed with insecticide, the residual effect of the insecticide fades over time and the process of vector return can begin [7]. After triatomine vectors colonize the area again, re-emergence of *T. cruzi* transmission may occur, increasing the risk for humans. Thus reinfestation and re-emergence of transmission are threats to vector control programs and prevention of Chagas disease.

Many species of triatomine bugs are able to transmit *T. cruzi* [8], but in the southern state of Arequipa, Peru, *Triatoma infestans* is the only insect vector for the parasite [9]. In 1999 the Ministry of Health (MoH) of Peru began systematic campaigns of insecticide spraying in areas affected by triatomines [10]. These campaigns, like many in Latin America, were conducted without comprehensive information on the extent of *T. cruzi* transmission in the area [10].

In the district of La Joya, Arequipa, our study team found that parasite transmission was interrupted in 1996 [11]. However, in the years leading up to 2008, dwellers of La Joya reported vector reinfestation in the area. In 2008, the MoH conducted an insecticide spraying campaign and, in collaboration with the MoH, our study team captured 2,070 triatomine vectors in human dwellings and 7,487 triatomine vectors in peridomestic areas. Interestingly, 96.7% of *T. cruzi*-carrying triatomine insects were captured in peridomestic areas (unpublished data). We also found that out of more than 500 humans 18 years or younger only 5 were infected in this area [11]. These findings led to the hypothesis that after vector reinfestation was established in La Joya, *T. cruzi* transmission was initiated in the peridomiciliary areas, where several domestic animal species can be found.

T. cruzi can infect a variety of animals, and several species have been identified as reservoirs and/or carriers of the parasite. Wild mammals such as opossums, raccoons, skunks, armadillos, mice, rats and other rodents have been reported as *T. cruzi* reservoirs [12-14]. Among domestic animals, dogs have been implicated in several studies as reservoirs of *T. cruzi* [15-18] and may play an important intermediary role in the transmission of the parasite to humans [19].

Because infection of dogs with the parasite occurs by vectorial transmission as well as by the oral route (dogs eating insects or infected mammals) [20,21], and taking into account that triatomine insects show a preference to feed on dogs [22], it is likely that in the process of reemergence of *T. cruzi*, dogs may become infected earlier than humans. In addition, dogs tend to live more proximate to humans compared to other animals, such as cattle or poultry. Therefore, identification of infected dogs and the factors that promote or hinder their infection is necessary for preventing human infection and describing the potential role of dogs as early indicators of re-emerging transmission. The objectives of this study were to (i) estimate the seroprevalence of *T. cruzi* in domestic dogs in an area where *T. cruzi* transmission is re-emerging after insecticide spraying, and (ii) to characterize the spatial association of infected dogs with the distribution of *T. cruzi*-infected *T. infestans* in an area of re-emergent *T. cruzi* transmission.

3.3. Methods

3.3.1. Ethical statement

Universidad Peruana Cayetano Heredia granted ethical approval for animal inclusion in the study. All protocols that involved animals were reviewed and approved by the Institutional Animal Care and Use Committee.

3.3.2. Location and study population

The study was conducted in the village of La Joya, which is located 30.7 km west (Euclidean distance) of the city of Arequipa, the capital of the state of the same name. La Joya sits at an altitude of 1617 m, has an average temperature of 18°C (range 10°C to 35°C), humidity ranging between 20% and 85%, and a marked rainy season. The residents of La Joya raise domestic animals for various uses. In addition to livestock and poultry, many families also keep dogs for protection and/or company, but not for hunting, which might expose dogs to *T. cruzi*-infected animals. The study area is historically endemic for Chagas disease; in 1996 the MoH conducted indoor residual spraying with insecticide for triatomine vector control. Over the 12 years that followed, insect reinfestation in the area led to a reemergence of *T. cruzi* transmission [11], and the area was treated again in 2008.

3.3.3. Study design and collection of biological data

Canine data

In July 2008, in coordination with the MoH we conducted a cross-sectional study in Villa La Joya, a community within La Joya district. A house-to-house animal census to collect age and sex of dogs and to assign a unique code to each animal was performed. Following the census, field veterinarians collected blood samples (1-3 cc) by phlebotomy from the saphenous or cephalic veins. Blood samples were processed in our local laboratory in Arequipa. Samples were centrifuged, and serum was aliquoted and stored at -20 C. Sera was sent to Lima and serological diagnosis, using an in house enzyme-linked immunosorbent assay (ELISA) previously standardized for dogs in Peru, was performed in the Research and Development Laboratory in Universidad Peruana Cayetano Heredia.

Entomological data

In August 2008, the MoH carried out a vector control campaign to spray insecticide in the district of La Joya, Arequipa, Peru. During the application of the insecticide in houses triatomine vectors are repelled by the chemical and emerge from their hiding places. In collaboration with the MoH, field workers followed the sprayers and collected triatomine insects from Villa La Joya. Each house was searched for triatomine insects for one person-hour. The rooms or animal pens where the search took place were given a unique code for identification. For purpose of analysis, all vectors caught in a single room or animal pen were assumed to comprise a single colony. Identified vectors were sent to our local laboratory in the city of Arequipa for parasitological analysis. Infection with *T. cruzi* was determined by direct microscopic observation. Briefly, a few drops of triatomine rectal contents were obtained by application of abdominal pressure, diluted with saline solution, and compressed between a glass slide and cover slip. The presence of mobile parasites in 50 microscopic fields was evaluated at 40X magnification.

Spatial data

The geographic location of the 258 houses in the study area, the rooms within those houses, and the animal pens were mapped comparing satellite imagery in

Google Earth ™ [23] to field maps drawn by our fieldworkers. Data from dogs were linked to the house location and triatomines data were linked to the animal pens or rooms where they were found. We implemented a Geographic Information System (GIS) in ArcGIS 9.0 [24] to link dog and triatomine data and to select datasets for analyses.

3.3.4. Statistical methods

Household locations were mapped to visualize the distribution of sampled, unsampled, and seropositive dogs. The location of pens and rooms where entomological surveys were conducted were also mapped to visualize the distribution of surveyed enclosures, infested enclosures, and *T.cruzi*-infected colonies. The ratios of the spatial intensity of seropositive dogs versus seronegative dogs and positive insect colonies versus negative insect colonies were evaluated with quartic kernels with bandwidths of 100 meters, the average length of a city block in La Joya. Spatial clustering of seropositive dogs and infected insect colonies was assessed using the K function difference [25]. Subsequently, we used a GIS to determine which dogs lived within 20m, 30m, 40, 50m, and 60 of *T. cruzi*-infected triatomines. This dichotomous variable (live or not live proximate to triatomines infected with *T. cruzi*) under different distances was used as an explanatory variable to examine serologic status of dogs using multivariate logistic regression. We used Akaike and Bayesian Information Criteria (AIC, BIC) for model selection and to determine which of these distances was the best fit. The total number of triatomines and the number of infected triatomines found in the dog's household were also evaluated as explanatory variables for dog seropositivity to *T. cruzi*. Age and sex of dogs were included in the models as adjusting variables. Multivariate logistic regressions and exploratory spatial data analyses were performed with R [26], and all statistical analyses were evaluated with a significance level of 0.05.

3.4. Results

One hundred and seventy-five dogs lived in the study area and were distributed across 150 households. We obtained blood samples from 154 dogs distributed in 127 households (sampled dogs = 88.0%; household participation rate = 84.7%). Increasing dog age was associated with lower participation in blood sampling. The sampled dogs were distributed over the study area and participation did not appear to be aggregated in a particular area in this study (Figure 2.1). Of 258 households in the study area, 253 (98.1%) participated in the entomological survey.

We found 19 seropositive dogs (seroprevalence = 12.3%), all of them 9-month old or older, 106 triatomine infested houses (41.9%), and 45 houses infested with *Trypanosoma cruzi*–carrying triatomine insects (17.8%). Seropositive dogs and insect colonies infected with *T. cruzi* were concentrated in certain parts of the study area (Figures 2.1 and 2.2), and there was some overlap of seropositive dogs and *T. cruzi*-infected colonies. An analysis of spatial odds ratios showed that seropositive dogs occur with greater intensity in certain areas of the field of study compared to the intensity of the negative dog population (Figure 2.3). Also in Figure 2.3 we observe that the areas of higher risk for dogs are close to or contain infected vector colonies. The clustering level measured by the K-function difference showed no clustering of seropositive dogs (Figure 2.4). By contrast, insect colonies infected with *T. cruzi* did show significant clustering between 10 and 75 meters (Figure 2.5).

The characteristics of the dogs according to their proximity (within 50m) to *T. cruzi*-infected triatomines are presented in Table 2.1. Nineteen of the 154 dogs were seropositive (12.3%) and the seroprevalence was higher in dogs within 50 meters of positive triatomines. Dogs proximate to triatomines infected with *T. cruzi* were significantly younger than dogs far from infected triatomines (3 years, 1 month vs. 4 years, 6 months). Overall most dogs were males (73.4%); this proportion did not vary between dogs proximate or far from infected triatomines.

We restricted the regression analyses to dogs greater than 9-months old. Based on the AIC and BIC values (Table 2.2) we report the results of the regression models when a distance of 50m was used as the cutoff for proximity between a seropositive dog and an infected colony. The association between canine seropositivity and presence of positive triatomines within 50 meters of the house was higher in the multivariate analysis compared to the univariate analysis, but in both cases the effect size was important and the association was statistically significant (Table 2.3). Dog age was statistically significantly associated with seropositivity in the univariate and multivariate model, but the effect size was small in both cases. Only the presence of *T. cruzi*-infected triatomines within 50 meters of the house and canine age were statistically associated with seropositivity in dogs (Table 2.3). The number of triatomines or number of positive triatomines captured in the dog's household did not show any association with the serologic status of the dog, either in the univariate or in the multivariate model.

3.5. Discussion

We documented here a relatively high prevalence of *T. cruzi* in dogs in an urban area of Arequipa, Peru. The human population in our study area was also studied in 2008 [11]. Of 125 children under-5 sampled in the same peri-rural community of La Joya, Arequipa during the same year only 1 was seropositive [11] and the child was born from a seropositive mother. That finding itself could have been enough to identify the area as free of vectorial transmission after spraying. However, in this study a high seroprevalence of *T. cruzi* in dogs (12.3%) was found. The area was treated with insecticide in 1996, 12 years before this study was conducted, but only three of the seropositive dogs were 12 year or older, ruling out that the high seroprevalence in dogs was the result of vector transmission prior to 1996. Congenital transmission was also considered, but all the seropositive dogs were 9-months or older and the odds of being seropositive increased with time after that age. Other studies have suggested the potential of dogs as proxies to determine human seroprevalence [27] and to detect *T. cruzi* transmission in rural areas [28]. Triatomine vectors show a strong preference to feed on dogs over other species [22], and oral transmission in domestic animals (ingestion) contributes substantially to the overall transmission of the parasite [29]. These characteristics may make dogs good markers to detect the reintroduction of the parasite to an area before the parasite reaches the human population.

The seroprevalence in dogs was very similar to that estimated by Tustin et al. for humans in the same study area (13.4%) [30]. We detected significant clustering of *T. cruzi*-infected colonies, but we did not detect clustering of seropositive dogs, similar to what Delgado et al. [11] found in seropositive humans of the same study area. Also, their results suggest that infection in humans occurred before spraying in 1996, and is not spatially related to the location of infected *T. infestans* in recent times. On the other hand, we estimated that the odds of being seropositive to *T. cruzi* for dogs living proximate to infected triatomines was 5.7 times greater than the odds for dogs living far from infected triatomines after adjusting for age and sex. Moreover, the strong spatial association observed in this study suggests recent transmission in those areas where seropositive dogs were found. Even though only nineteen seropositive dogs were found in the area, the results in the multivariate analysis were statistically significant, suggesting this relationship would hold in similar settings.

There were some confounders that should be evaluated to assess the validity of estimates. The association between non-participation in sampling and increasing age of dogs could be explained by aggressive behavior in older dogs as reported by field workers, preventing handling and taking of blood samples. This participation rate differential by age could have biased our results; however, given the positive association found between age and seropositivity, if all dogs had been sampled, we would expect to see an even greater association, assuming that participation rate is not associated with seropositivity. The high proportion of male dogs in the sample population is explained by anecdotal conversations with local residents who state they prefer male dogs, as they are considered to be better guard dogs and they do not have to worry about unexpected pregnancies in their animals, a common problem in rural areas of Peru where animals are rarely or never spayed. The possible interaction between time of exposure to positive triatomines and proximity to positive triatomines was assessed. In the study area, dogs are typically acquired as puppies and rarely change owners; therefore, age is a good surrogate for time of exposure. We did not observe changes in the estimated coefficients and the addition of this interaction term did not improve the multivariate model (Likelihood Ratio Test post-estimation: $p = 0.6221$).

There is a wide range in canine seroprevalence in The Americas, and the seroprevalence of 12.3% estimated in La Joya, Arequipa lays in the middle of that range [16,31-36]. The ELISA we used to estimate seroprevalence was compared to a TESA-blot assay, a confirmatory test for inconclusive results. We found a concordance of 0.87 using the Kappa test. However, the lack of a gold standard and estimates of sensitivity and specificity are limitations for our results.

Our study considered proximity to *T. cruzi*-infected triatomines as an explanatory factor for seropositivity in dogs, but the potential of dogs for transmitting *T. cruzi* to nearby triatomine colonies should not be overlooked. The potential role of dogs in the transmission of *T. cruzi* has been suggested by several studies based on seroprevalence results. Serological canine surveys in Paraguay and Argentina have found a higher prevalence of infection in dogs than in other domestic animals [15-17]. As mentioned above, Gürtler et al [16] found that *T. infestans* strongly prefers to feed on dogs and cats compared to chickens, which would increase the chances of vectors acquiring the parasite in areas with infected dogs. Another study from Argentina reported an association between infected dog ownership and increased risk of Chagas disease in children [19]. Finally, it has been reported that dogs living within the same area can be infected with *T. cruzi* from different discrete strains of the parasite revealing the versatility of dogs as reservoirs of the parasite [37].

The number of bugs and the number of triatomine insects infected with *T. cruzi* have been described as risk factors for infection with *T. cruzi* in humans [38-41]. However, in our analysis, these factors showed neither an important effect size coefficient nor a statistically significant association with seropositivity in dogs. The study community is considered a rural town, but the distribution of households does not follow the typical scattered pattern of other rural areas [42]. The houses in Villa La Joya have an area of 300m^2 on average and are in close proximity to each other, grouped into blocks that are arranged similarly to urban blocks. The rustic house materials and the poor conditions of the houses are probably not an efficient barrier to the mobility of triatomines between houses. A triatomine captured in one house may have originated and fed on humans or animals living in neighboring houses. The strong association between seropositive dogs and triatomine insects infected with *T. cruzi* found in areas surrounding the dog's household reinforces this hypothesis.

Indoor insecticide spraying is the primary intervention for vector control of Chagas disease in the Southern Cone [6,43] and the detection of positive triatomine insects has been used to identify high-risk areas [38]. In low-resource areas, which comprise most of the areas affected by Chagas disease, dogs could also be use to detect areas with ongoing or re-emergent transmission and prioritize actions for early intervention. Sanitary programs focused on dogs, such as anti-rabies vaccination campaigns, have the advantage of congregating many dogs in a few points where MoH personnel have access to them. Taking blood samples during these campaigns and determining infection in dogs with pooled blood samples could potentially be a more cost effective use of resources.

If dogs are used as sentinels, considerations for handling infected dogs have to be in place to guarantee reduction of transmission. Elimination of positive dogs would be the most effective measure to halt transmission; however, people who regard their dogs in high value would not participate of these sentinel surveillance programs. Given the importance of dogs in the epidemiology of Chagas disease and their reservoir competence for *T. cruzi* [32,44,45] attempts to disrupt the transmission cycle in dogs should be considered. Some of these attempts have found increased mortality of *T. infestans* that feed on dogs treated with topical insecticide [46,47] and reduced feeding and engorging rate in *T. infestans* that live with dogs using deltamethrin-treated collars [21,48]. Overall, these studies found high variability in their results and suggest that measures to reduce transmission from and to dogs need more development.

3.6. Conclusions

Seroprevalence of dogs was high in an endemic area of Peru after 12 years of insecticide spraying. Dogs may be useful sentinels to detect reemergence of *T. cruzi* transmission and should be considered in surveillance programs. *T. cruzi*-seropositive dogs are spatially associated with the presence of *T. cruzi*-infected *T. infestans* colonies. *T. cruzi* transmission foci detection, determined by entomological surveys, could direct targeted insecticide treatment and prompt the testing of canine reservoir hosts in the proximity of positive vector colonies. Prevention efforts can be directed at interrupting the re-initiation of *T. cruzi* transmission from dogs as the primary reservoir hosts. Prior studies have explored topical insecticides as means of halting the vector-host (canine) transmission process with variable results. Finally, given the strong spatial association between seropositive dogs that are near *T. cruzi*- infected triatomines, canine serological surveys nested within other programs such as rabies-vaccine campaigns, should be evaluated as cost-effective strategies to identify areas were vectorial transmission is reemerging or high-risk areas within endemic zones [31].

3.7. Acknowledgements

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Table 2.1. Characteristics of dogs and serology by proximity to *T. cruzi*-infected triatomines

	Dogs ≤ 50m of	Dogs > 50m of	Total	p-value
Seropositivity, n	17 (15.2)	2 (4.8)	19 (12.3)	0.08[a]
Age in years, mean	3.1 (0.3)	4.6 (0.6)	3.5 (0.3)	0. 01[b]
Sex, n (%)				
Males	33 (78.6.)	80 (71.4)	113 (73.4)	0.37[a]
Female	9 (21.4)	32 (28.6)	41 (26.6)	

Evaluated with [a] Chi-squared and with [b] t student tests; n:.count; %: percentage; y SE: standard error

Table 2.2. Akaike and Bayesian Information Criteria for different proximity cut-offs

	20m	30m	40m	50m	60m[a]
AIC	116.24	115.91	113.53	110.51	105.34
BIC	128.36	128.03	125.65	122.63	1.12 – 28.74

[a] 60m offered the lowest AIC and BIC but was omitted from the final model based on linearity.

Table 2.3. Associated factors to seropositivity to *T. cruzi* in dogs 9-month old or older

	Unadjusted Model			Adjusted Model [a]		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
Live ≤ 50m of an infected triatomine	4.69	1.03 – 21.44	0.04	5.82	1.12 – 28.32	0.03
Age in years	1.12	0.97 – 1.29	0.01	1.16	0.99 – 1.43	0.06
Sex (males)	1.08	0.36 – 3.28	0.88	1.15	0.34 – 3.65	0.82
Number of triatomines captured in the household	0.99	0.98 – 1.01	0.47	1.00	0.98 – 1.01	0.57
Number of *T. cruzi*-infected triatomines in the household	1.01	0.83 – 1.46	0.49	1.14	0.85 – 1.46	0.37

95% CI: 95% confidence interval. [a] Adjusted by sex, age

Figure 2.1. Map showing the canine population

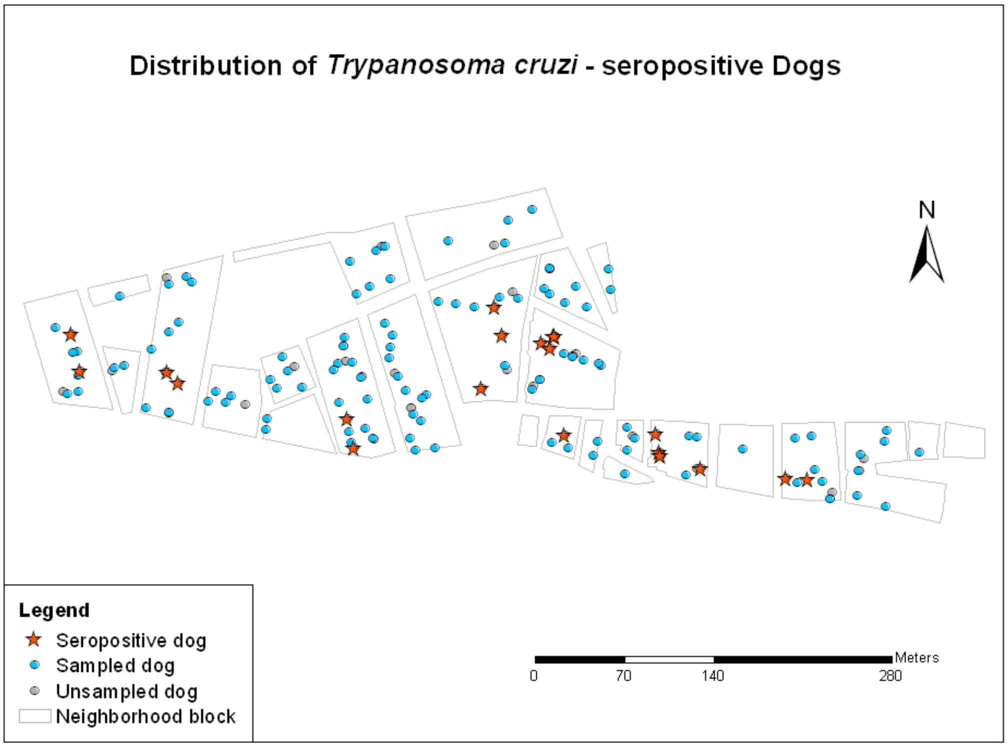


Figure 2.2. Map showing the vector colonies

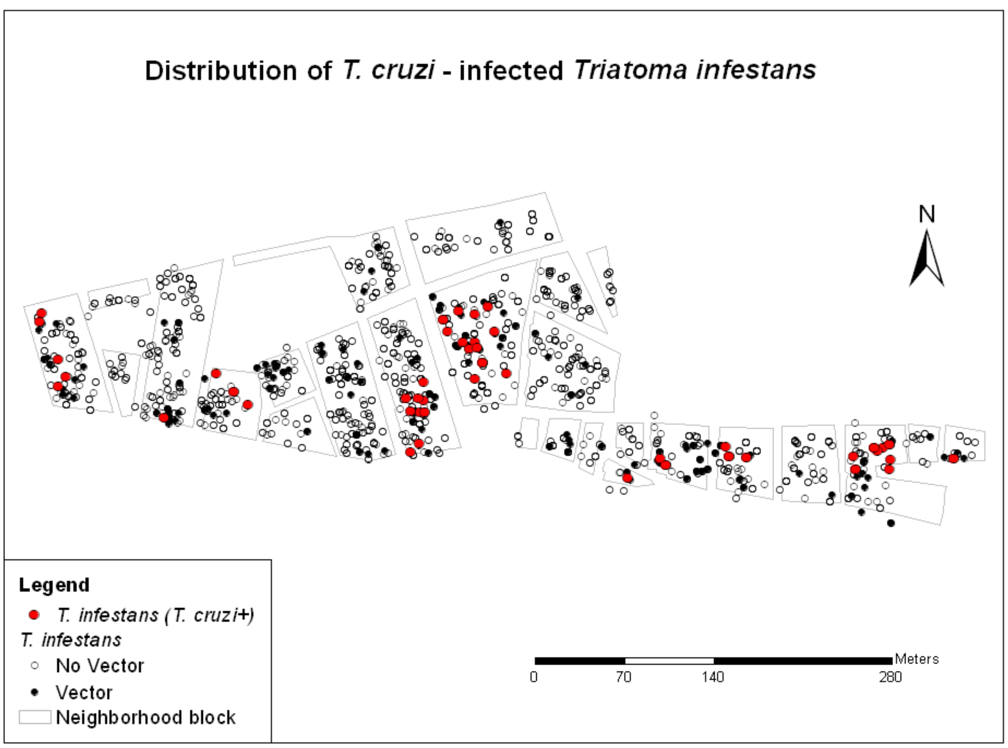


Figure 2.3. Spatial odds ratios of seropositivity for *Trypanosoma cruzi* in dogs. UTM coordinates.

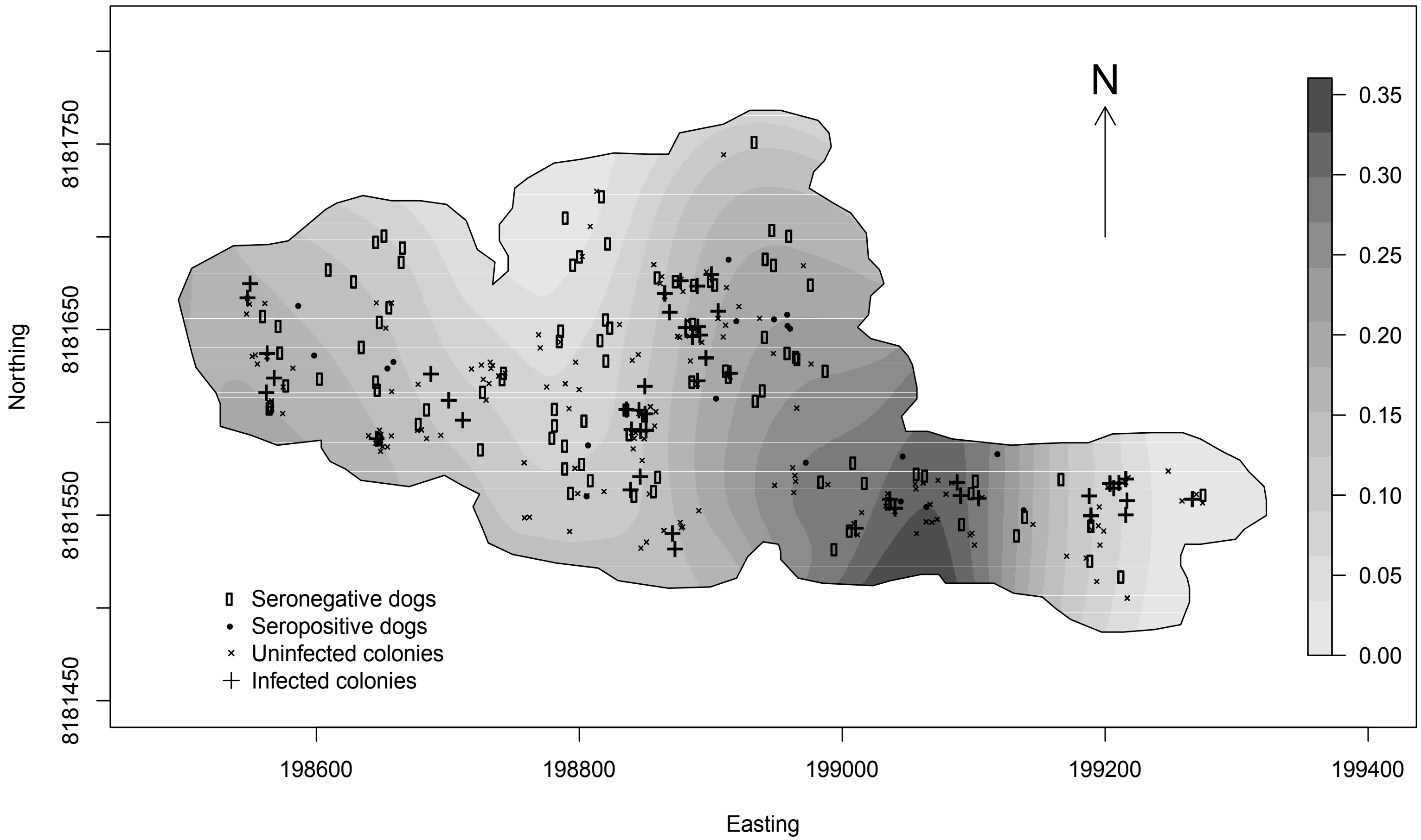


Figure 2.4. Clustering of seropositive dogs

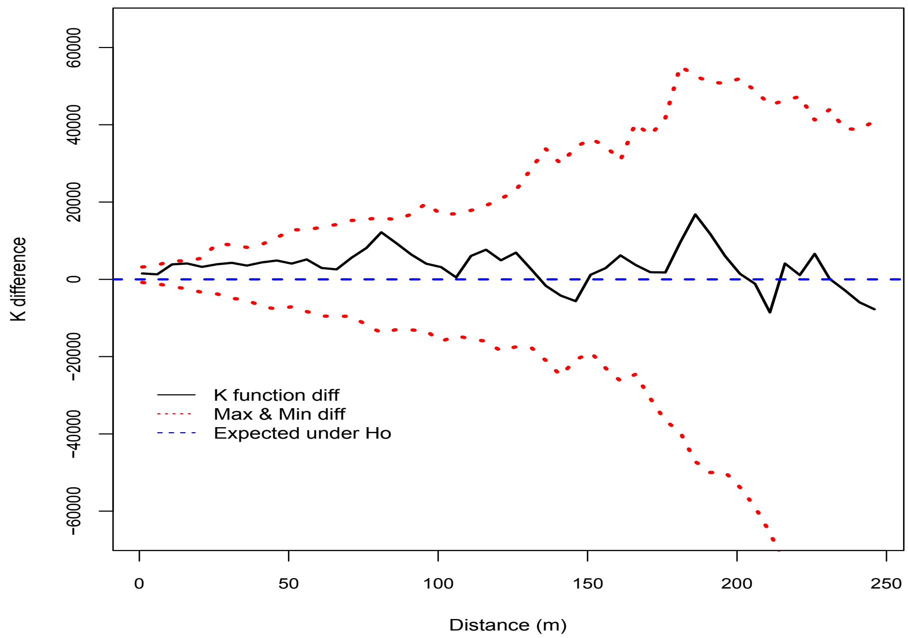
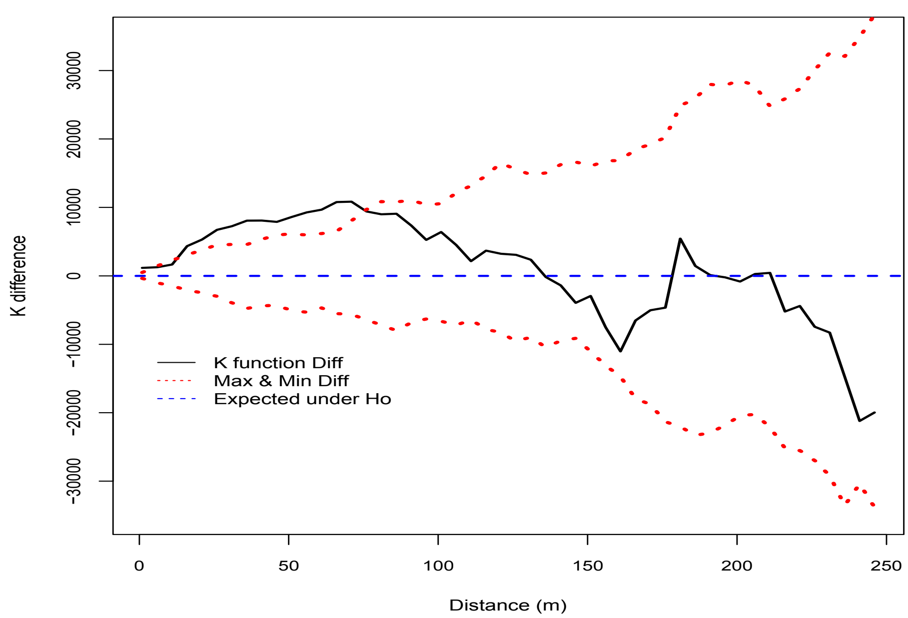


Figure 2.5. Clustering of infected vector colonies



4. Host-seeking behavior and dispersal of *Triatoma infestans*, a vector of Chagas disease, under controlled conditions.

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4.1. Abstract

Background: Chagas disease affects millions of people in Latin America. The control of this vector-borne disease focuses on halting transmission by reducing or eliminating insect vector populations. Most transmission of *Trypanosoma cruzi,* the causative agent of Chagas disease, involves insects living within or very close to households and feeding mostly on domestic animals. As animal hosts can be intermittently present it is important to understand how host availability can modify transmission risk to humans and to characterize the host-seeking dispersion of triatomine vectors on a very fine scale.

Methods: We used a semi-field system with motion-detection cameras to compare the behavior of vector populations in the constant presence of hosts (guinea pigs), and after the removal of the hosts. We had 2 tanks: a control tank (constant guinea pigs) and an intervention tank (guinea pigs removed). We observed the experiment for 5 weeks and repeated the experiment 3 times. We counted every three days how many insects remained in the original refuge to determine the emigration rate and evaluated this rate with three different Poisson models. We also counted the motion-activated snapshots to assess the level of activity with a hierarchical linear model.

Results: The emigration rate – net insect population decline in original refuge – following host removal was on average 19.7% of insects per 10 days compared to 10.2% in constant host populations (p=0.029). However, dispersion of *T. infestans* occurred in both directions, towards and away from the initial location of the

hosts. The majority of insects that moved towards the original location of guinea pigs remained there for 4 weeks. Oviposition and mortality were observed and analyzed in the context of insect dispersal, but only mortality was higher in the group where animal hosts were removed (p-value < 0.01).

Conclusions: We discuss different survival strategies associated with the observed behavior and its implications for vector control. The removal of domestic animals in infested areas increases vector dispersal, possibly towards nearby human sleeping spaces.

Keywords: *Triatoma infestans*, Chagas disease, insect dispersal, host-seeking behavior.

4.2. Introduction

Chagas disease, a vector-borne disease caused by the parasite *Trypanosoma cruzi*, affects from 7 to 8 million people in The Americas [1] . The vast majority of people infected are not detected [2,3], and when the disease manifests clinically it cannot be reversed and can be fatal [2-4]. In addition to the inability to detect early cases and the lack of treatment in advanced stages, currently, there are no vaccines available to prevent the disease [5]. Therefore, efforts to halt transmission are crucial in endemic areas to reduce the burden of disease [6].

Most control programs aimed to halt *T. cruzi* transmission focus on reducing the insect vector populations [6]. In the Southern Cone of South America, transmission of *T. cruzi* is mostly driven by *Triatoma infestans* living within or very close to households, at times feeding on humans, but feeding mostly on domestic animals [7,8]. In urban areas and densely populated rural areas, the proximity between animal corrals and human bedrooms may allow for dispersal of vectors from animal enclosures into human houses.

Several studies have reported the presence of guinea pigs in houses as a risk factor for triatomine infestation in endemic areas [9-11]. In Arequipa, Peru, an area where Chagas disease is an emerging and re-emerging problem, the presence of domestic guinea pigs increases the odds of triatomine-insect infestation by 1.69 times and the triatomine density by 2.4 times [12]. In this setting, guinea pigs are raised in small numbers in backyards, on top of roofs, and inside the houses as a source of protein. Many factors can lead to changes in the distribution and presence of domestic animals of different species. Because guinea pigs are typically fed with alfalfa, the price of which fluctuates widely, and because of their small number in a corral, guinea pigs are often withdrawn during certain parts of the year and the corral left empty. This is a presumed catastrophic event for local triatomine populations relying on those animals as a source of blood meals. When hosts are removed from corrals the triatomine insects that live, reproduce and feed on them might either leave, or stay to wait for a new wave of hosts. If the triatomine vectors leave their nests in search of new hosts, the removal of guinea pigs likely implies a sudden and important rise of the risk for the human populations.

The dispersal behavior of different triatomine vectors has been studied from different perspectives. *T. infestans* uses two types of locomotion for dispersal: flying and walking. In Argentina most *T. infestans* were found walking in infested areas, but a number of them were captured flying [13]. To add complexity to the locomotion patterns observed in *T. infestans*, it has been reported that the starting time of flight shows wide variability based on climatic and individual factors [14] and that *T. infestans* do not fly above 2,750 m [15], an important feature in the highly populated cities of the Andes with altitude in this range such as Arequipa in Peru and Cochabamba in Bolivia. For other triatomine species, light might attract insects into houses [16], and street lights have been associated with increase infestation in houses [17]. Some studies have reported patterns of walking dispersal related to host seeking, mainly by isolating the effect of chemical [18-20] and physical cues [21-24]. It is not yet known how triatomine insects disperse in the event that their food source completely disappears from their surroundings. In order to determine how the vectors behave under these circumstances, we characterized the initial dispersal behavior of triatomine insects at small-scale area when the only source of blood meals is withdrawn from the environment, and compared it to an identical environment in which the host population remains constant.

4.3. Methods

4.3.1. Ethical statement

The Institutional Animal Care and Use Committee (IACUC) of Universidad Peruana Cayetano Heredia reviewed and approved the animal-handling protocol used for this study (identification number 60942). The IACUC of Universidad Peruana Cayetano Heredia is registered in the National Institutes of Health at the United States of America with PHS Approved Animal Welfare Assurance Number A5146-01 and adheres to the Animal Welfare Act of 1990.

4.3.2. Experimental design

In order to understand the dispersal of *T. infestans* after blood-meal sources are removed, we conducted an experiment in a semi-field system with motion-detection cameras. In two 10-foot long glass tanks with a glass-walled maze in the middle area we placed a cage with two guinea pigs at one extreme of the tanks. A cardboard box with corrugated paper to increase internal surface (*the primary refuge*) containing 60 triatomine insects was placed in each tank proximal to the guinea pig cage. An identical cardboard box (*the secondary refuge*) was placed at the extreme of the tank opposite to the guinea pig cage and the primary refuge (Figure 1). After a week of cohabitation, the guinea pigs from one of the tanks were removed (*time of intervention*); this tank was designated the *intervention tank* and the tank with constant presence of guinea pigs was designated the *control tank*.

We defined six discrete locations across each experimental tank: quadrants 1-4 and the primary and secondary refuges. Quadrant 1 refers to the area occupied by the guinea pig cage at one extreme of the tank; the primary refuge is proximal to this quadrant. Quadrant 2 refers to the area immediately surrounding the primary refuge and the beginning of the maze. Quadrant 3 refers to the middle portion of the maze. Quadrant 4 refers to the final portion of the maze and the area immediately surrounding the secondary refuge, which was placed at the extreme of the tank (Figure 1). The insects were free to move throughout all areas of the tank.

During the experimental period, we counted the number of *T. infestans* in these six different areas of the intervention and control tanks, video-recorded their movements, and estimated their level of activity by motion-activated snapshots. The experiment was repeated three times. We used a HOBO® data logger to record temperature and relative humidity every 10 minutes during the three replicates of the experiment.

4.3.3. Animals

We used a total of 12 one-month-old female guinea pigs from a local farm free of triatomine infestation and a total of 360 triatomine insects that were raised in a large triatomine colony originated from Arequipa, Peru. In order to form the 60-triatomine groups for each tank and each repetition, we chose 10 triatomine insects from the following six groups: 2nd, 3rd, 4th, 5th instar, female and male.

4.3.4. Data collection

We used a video camera system to observe and record two types of files; snapshots of each quadrant of each tank every 10 seconds and snapshots of a quadrant where any movement was detected to a maximum of two motion-activated snapshots per second. Because of the continuous movement of guinea pigs in quadrant 1, we only used snapshots from the other 3 quadrants where activation of snapshots was solely related to triatomine movement. Because triatomines are nocturnal, we used red-light bulbs between dusk and dawn to provide illumination for the cameras. Red light has been shown previously to not affect triatomine movement [25,26], and it has been used to observe other nocturnal insects [27,28]. To confirm the lack of disturbance of red light in the dispersal of *T. infestans* we conducted a pilot with three different light colors: white, green, and red. We illuminated a 2-square-foot-area tank containing 20 *T. infestans* and observed their behavior. Under white and green light the triatomine insects stayed on the borders of the tank and only moved along the borders, a behavior consistent with negative phototaxis. Under red light the triatomine insects moved all over the tank surface without showing any pattern that would suggest light disturbance.

We also visually examined each tank at around 10 AM every three days and the day before and after removing guinea pigs. We counted the number of triatomine insects in each quadrant and box and registered their sex or developmental stage and their nutritional status. The nutritional status was measured in a scale from 1 to 4, and it was based on a qualitative determination of blood reserves in the mid gut developed by Montenegro in 1983 [29]. During these in-person observations, we recorded the number of dead insects and eggs laid in each quadrant or refuge. We withdrew the eggs from the tanks upon observation, but left dead insects where they were found.

4.3.5. Statistical analyses

Analysis of videos: We compared the activity of triatomine insects during the observation periods by analyzing the daily total number of motion-activated snapshots per tank taken by the cameras. We used a hierarchical linear model with random intercept to model the average number of motion-activated snapshots depending on the intervention. We used the recorded temperature and relative humidity every 10 minutes to calculate the median daily temperature and the median daily relative humidity to include them as regressors.

Analysis of counts of insects: To model the differential dispersal of triatomine populations in the intervention and control tanks we used a Poisson regression on the insect counts in the primary refuge over time depending on the presence or absence of hosts. We first considered a simple Poisson regression. Second, we considered a hierarchical Poisson regression with random intercept to account for different counts in the refuge at the time of intervention. Finally, to allow random variation of the rate of emigration, or population decline of the primary refuge, between repetitions, we used a hierarchical Poisson regression with random intercept and slope. We also included as regressors the median daily temperature and the median daily relative humidity. The fit of the alternative models to the data was compared with Akaike's Information Criterion (AIC).

We plotted the observed number of triatomine insects in each quadrant and boxes over time. We also plotted the observed number of triatomine insects in the box close to the guinea pig cage as a function of time and the predicted number obtained from the hierarchical Poisson regression with random intercept, the model with lowest AIC. The number of triatomine insects that were found in the original insect refuge the day before the intervention was compared with chi-squared test.

Death and oviposition: Finally, we evaluated the number of eggs found over the study area and compared the number of dead insects between intervention and control tanks with a log-binomial regression. All graphs and analyses were produced in R [30] and all estimates were analyzed at α level = 0.05.

4.4. Results

Across the three repetitions, we observed a similar pattern of emigration of insects; the number of triatomine insects in the primary refuge decreased faster in the intervention tank than in the control tank (Figures 2 and 3). We also observed that triatomine insects in the intervention tank leaving their primary refuge dispersed in both directions, towards and away from the empty guinea pig cage (Figure 2). In the control tank the number of triatomine insects in the primary refuge reduced slightly over time and those triatomines that emigrated did it always towards the secondary refuge.

When we evaluated dispersal by developmental stage and sex, we observed more complex patterns. The most remarkable finding is that females, independently of the tank, started dispersal immediately after they were placed in the experimental area. A proportion of the females quickly found the secondary refuges and stayed there. A related observation was the presence of eggs in the primary and secondary refuges and across the quadrants. We did not observe any pattern in the distribution of eggs over the study area, but the number of laid eggs increased exponentially over time in the three repetitions in both tanks as shown in Figure 5. The three Poisson regression models we used to quantify the rate of emigration of *T. infestans* from their primary refuge in our system estimated similar rates, with an average of 10.2% and 19.7% reduction of insects in the primary refuge per 10 days lapsed in the control group and the intervention group, respectively. This difference was statistically significant, with p-values between 0.029 and 0.036 depending on the model (Table 3.1). Overall, the AIC favored the hierarchical Poisson model with a random intercept (Table 3.1), suggesting that the counts of insects in the refuge at the time of intervention might be different between repetitions but the net emigration rates after the intervention are consistent across repetitions. Nevertheless, the heterogeneity in the number of triatomine insects in the primary insect refuge at the time of intervention was not statistically significant (chi-squared = 0.3748; df = 2; p-value = 0.83).

The dispersion of the data around the values predicted by the best model is presented in Figure 3. The nutritional status of triatomines found in the secondary refuge the day before intervention was not statistically different when we compared the intervention versus the control tank (Fisher's exact test p-value=0.39). We observed high variability of daily median relative humidity and low variability of daily median temperature as observed in Figure 6. These weather variables had neither an important effect size nor a statistically significant influence on the emigration rate.

In terms of observed level of insect activity, there were complex patterns in the intervention tank after guinea pigs were removed. The night after the guinea pigs were removed, we observed an elevated number of movements recorded by our system in the intervention tank compared to the control tank. Video 1 shows quadrant 1 and primary refuge of the intervention and control tanks between 11

PM and 2 AM the night after guinea pigs were removed. The video was created with the snapshots taken automatically every 10 seconds and clearly shows the high level of activity in the intervention tank. Over the observation period, the level of activity was higher in the intervention tank, and can be observed by comparing the spikes in Figure 4. The average number of motion-activated snapshots per day was higher in the intervention tank compared to the control tank by 11,186 snapshots across all three repetitions (95% CI: 4,653 – 17,720; p = 0.001). A one Celsius degree increase in the median daily temperature was associated with an increase of 5,068 motion-activated snapshots per day (95% CI: 1,317, 8,818; p-value=0.01), and an increment of one percentage point of relative humidity was associated with an increase of 302 motion-activated snapshots per day (95% CI: -49, 655; p-value=0.10). An observation related to the frequency of movements recorded by our system is that insect activity started on average 1 hour and 12 minutes earlier in the intervention tank compared to the control tank the night after hosts removal.

The probability of triatomine insects dying in the intervention group was 1.46 times higher than in the control group and this increase was statistically significant (95% CI: 1.10, 1.95; p-value=0.0098). Most dead triatomine insects were found in the primary refuge and no significant differences in mortality were observed by developmental stage or sex.

4.5. Discussion

Here we characterize the ex-situ dispersal of *T. infestans* after removal of sources of blood. The observed reduction of insect population in their primary refuge (emigration rate) was, on average, 19.7% over 10 days, and triatomine insects did not distribute randomly over the available area. Some of the insects remained in the primary refuge (close to the guinea pig cage), some migrated to the secondary refuge (far from the guinea pig cage), and some dispersed towards the empty guinea pig cage. The empty guinea pig cage was the only source of olfactory cues associated with blood sources, and also offered shelter to bugs.

In r/K selection theory, some species develop an r strategy that favors population growth rate (r) with low intra-species competition, and many offspring with low probabilities of survival, while others develop a K strategy, where population growth is limited by carrying capacity of their environment (K), few offspring are produced, all with a high probability of survival, and intra-species competition is high [31]. These two strategies also correspond to different dispersal patterns: a pure r-strategy would involve a continuous high level of dispersal that would minimize the impact of perturbations, and oppositely, a pure k-strategy would minimize dispersal under constant conditions, but may display significant dispersal in the face of perturbation. Active dispersal of triatomine insects has been mainly associated with seeking food and mating [32], but it might also be an r-species strategy to find and colonize new areas, maximizing the overall survival of the descendants in the case of unpredictable environments [33]. The significant dispersal to the refuge at the other side of the tank, even with blood sources at immediate proximity suggests that part of the dispersal of *T. infestans* is linked to an r-strategy type dispersal. The significant impact of the removal of the hosts on the dispersal rate confirms that there is a K-strategy type dispersal component for a mixed strategy in *T. infestans* dispersal.

We observed two opposite responses after guinea pigs were withdrawn; a proportion of insects stayed close to the guinea pig cage, while others migrated away. One explanation for the prolonged presence of triatomines close to the empty guinea pig cage is the continued presence of chemical attractants for triatomines. Specifically, urine [34], humid feces, or even humid scraps of pasture greens may attract triatomines [22]. The continued presence of the insects in the empty guinea pig cage may also be behavioral, as insects accustomed to feeding in that location that do not become engorged may wait for animals to return. Remaining near a previously present food source may be an advantageous strategy in areas where foraging for new food sources carries a high cost. Triatomine insects have a number of nocturnal predators such as geckos, rodents, and spiders, and diurnal predators such as chickens, dogs, and cats. In addition to the risks of predation, seeking new food sources might deplete the insects' energy stores and expose them to desiccation.

A large proportion of insects also migrated away from the empty cages. The two opposite responses observed might represent two different individual strategies that increase the individual's survivorship in the field in different ways; a passive-host-seeking individual's genes will pass along if they wait for food and new hosts come in a timely manner, and an active-host-seeking individual's genes will pass along if they are able to avoid all the risks outside of the refuge and successfully find new hosts. However, our experiment was not set up to trace kin relationships or to distinguish genetically-based variations from stochastically determined behaviors (bet hedging) [35,36], so we cannot make any conclusions in that regard.

Interestingly, the potential strategy associated with a passive food-seeking behavior (waiting in a high-probability-of-food zone) could explain some previous field observations by our team. In 4 rural communities of Arequipa, Peru, we conducted entomological surveys for triatomines in each animal corral in the area. Among 1762 animal corrals we found *T. infestans* in 294, and 104 of these infested corrals did not contain any animals. Those empty corrals might represent high-probability-of-food zones that are exploited by triatomine insects and should be considered in vector-control strategies.

The increased frequency of movements and the earlier start of insect activity observed in the intervention tank versus the control tank may be explained by active search for a host. Surprisingly, across the three repetitions this differential behavior started the same night that guinea pigs where withdrawn. The insects from both tanks had cohabitated with the guinea pig for one week prior so, assuming a similar biting rate to that previously reported [37], it is possible that most insects needed to feed the same night of guinea pig removal. It is also possible that triatomines can recognize the lack of a source of blood meals in the vicinity and may start searching hosts for the next meal, even if they do not need to feed for several days. Hosts might also be sources of heat for proper enzymatic activity and be sought by engorged or partially full triatomine insects to facilitate digestion [38].

We observed that in both tanks, control and intervention, triatomines were usually found in the extremes of the tanks during in-person observations (~10 AM). Ideal free distribution theory [39] proposes that animals know the quality of the patches (distribution of resources) where they move and will choose patches with higher quality. In our intervention tank, after removing guinea pigs, the quality of the quadrants in terms of food became the same; however, the presence of the refuges as well as the presence of the empty guinea-pig cage with feces, urine and scrapes of alfalfa provided cues as well as safe harbor for insects, making some quadrants more attractive than others. Thus, ideal free distribution might explain the similar distribution of *T. infestans* in areas far from and close to the location of blood-meal sources after removal of hosts.

We found *T. infestans* eggs scattered across the experimental tanks, without any clear pattern of dispersion. The absence of spatial pattern in the distribution of eggs might suggest a strategy to disperse eggs around the original colony, and could support the null hypothesis of the ideal free distribution of triatomine insect females over the experimental area. The importance of walking pregnant triatomine females was reported by Abrahan et al. in 2001 [40], and suggested that this type of locomotion in females is an adaptive strategy that allows for dispersal of many eggs. Dispersing eggs around the original colony increases the chances of at least one egg surviving and it is a preferred strategy as the intensity of predation increases [41]. Egg dispersal might also increase colonization success and help to avoid reaching carrying capacity if the colony only grows with a limited supply of blood meals or nesting space.

We did our best to maintain the intervention and control groups under identical conditions throughout the experiments. Despite our efforts, there were a number of insects that started dispersal in the intervention tank before guinea pigs were withdrawn in two of the three repetitions. The slight observed difference in the number of insects in the original refuge before the guinea pigs were withdrawn was not statistically significant for any of the three repetitions. For all repetitions the control and intervention tanks were switched, and in all cases the insects started the repetitions in a completely clean tank without residues from previous experiments that could have leaved traces of olfactory cues. One potential explanation we propose is a different individual guinea pig response to insect bites that would have prevented triatomines from completing a blood meal in a tank with more irritable guinea pigs and led them to look for other sources of blood meals. However, there was no statistical difference in the nutritional status of those insects found in the primary and secondary refuge before withdrawing the guinea pigs. A simpler explanation is that triatomine insects display a clear r-strategy, dispersing considerably even when they have reliable food sources and refuge.

We faced some limitations that should be taken into account when making inferences from our results to *in-situ* triatomine dispersion. We had a fixed number of triatomines by developmental stage and sex not reflecting the stable population distribution of *T. infestans* [42]. Patterns of dispersion, however, might be influenced by density and stage structure of vector colonies [43]. We kept a fixed number of guinea pigs across all repetitions, but the ratio of hosts to vectors might also influence dispersal patterns [44]. Also, the number of triatomine insects per tank reduced slightly during the experiment due to mortality, but we did not allow it to increase, as it could under field conditions. Changes in population size might affect dispersal, especially when the number of insects exceeds the carrying capacity of the environment. In addition, we observed our *ex-situ* system for only 28 days after the removal of the hosts. The dispersal of triatomines would certainly have continued past our period of observation, and different patterns could have emerged over longer time scales. In our system there was significant variation in relative humidity and small variation in temperature over time. A greater variability of temperature and relative humidity would be expected in field conditions across different ecotopes and in peridomestic areas, probably influencing the activity and the dispersal of *T. infestans*, in keeping with our observed increased activity associated with higher temperature. Finally, we purposely did not place attractants in the other site of the tanks such as secondary sources of blood meals in an effort to mimic situations in which migration would entail seeking an entirely new food source. In areas where animal corrals are close to one another or to rooms where humans sleep most migration might be directed to an easily detected area. Cues from proximal animals or humans would be detected soon after food seeking starts and then traces of those cues would lead the insects directly to the hosts [45]. It is likely then that the observed propensity to disperse when hosts are removed would be even higher in a field environment.

4.6. Conclusions

Our results represent triatomine dispersal in areas of low-animal-corral density and might, as well, represent initial dispersal of triatomine insects in areas of high-animal-corral density. While dispersal is important even in the constant presence of hosts, there is an important change in the dispersal pattern when hosts are removed. We observed two types of dispersion: close spatial association with original location of removed hosts, and dispersion seemingly at random far from the primary refuges and host locations. Further studies would help to determine the ecological meaning of these dispersal strategies, and could answer if behavioral patterns are the result of bet hedging. The removal of these animal hosts may lead to sudden infestations of surrounding areas by insects looking for other sources of blood meals, increasing the risk of *T. cruzi* transmission for humans in proximal areas. Further studies are needed to discern adequate strategies to limit *T. infestans* dispersal in these settings and the associated increase of transmission risk. Additionally, empty animal corrals may remain attractive to the vectors or be used by triatomines as hiding places and should be carefully considered in vector-control activities such as monitoring, insecticide treatment, and housing improvement [12,46-48].

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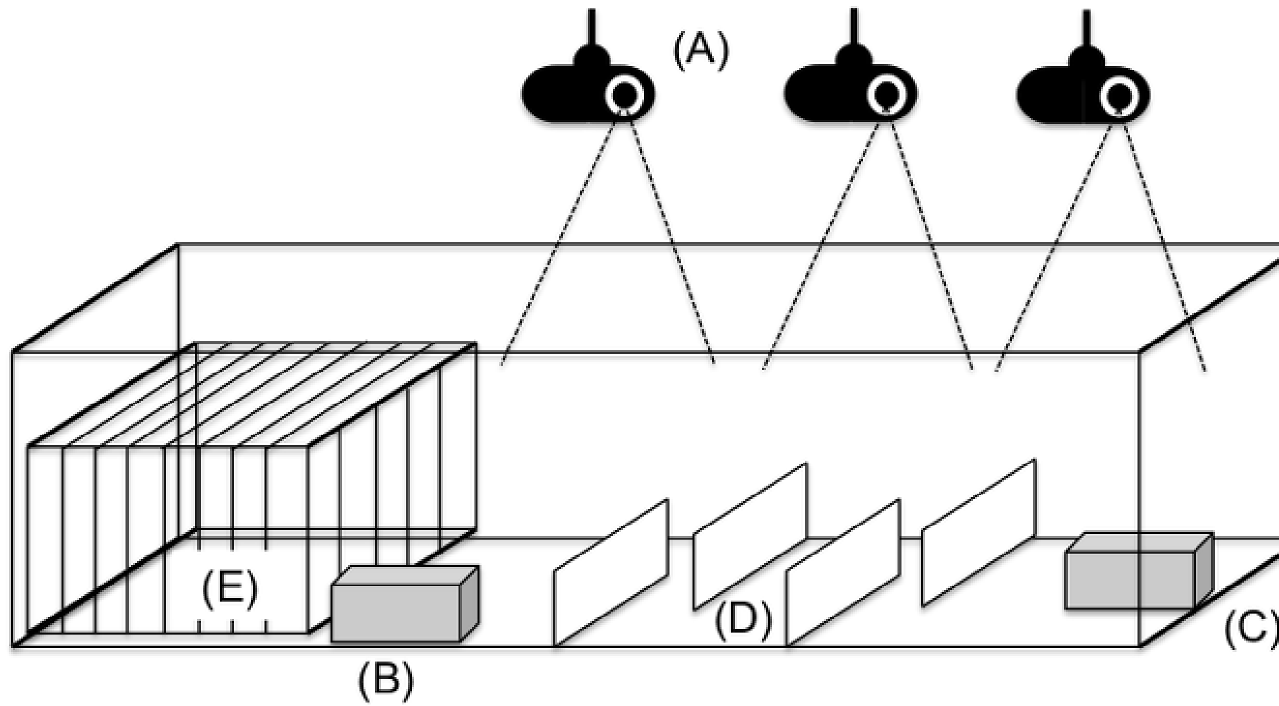
Table 3.1. Comparison of Poisson regression models to assess the emigration rate.

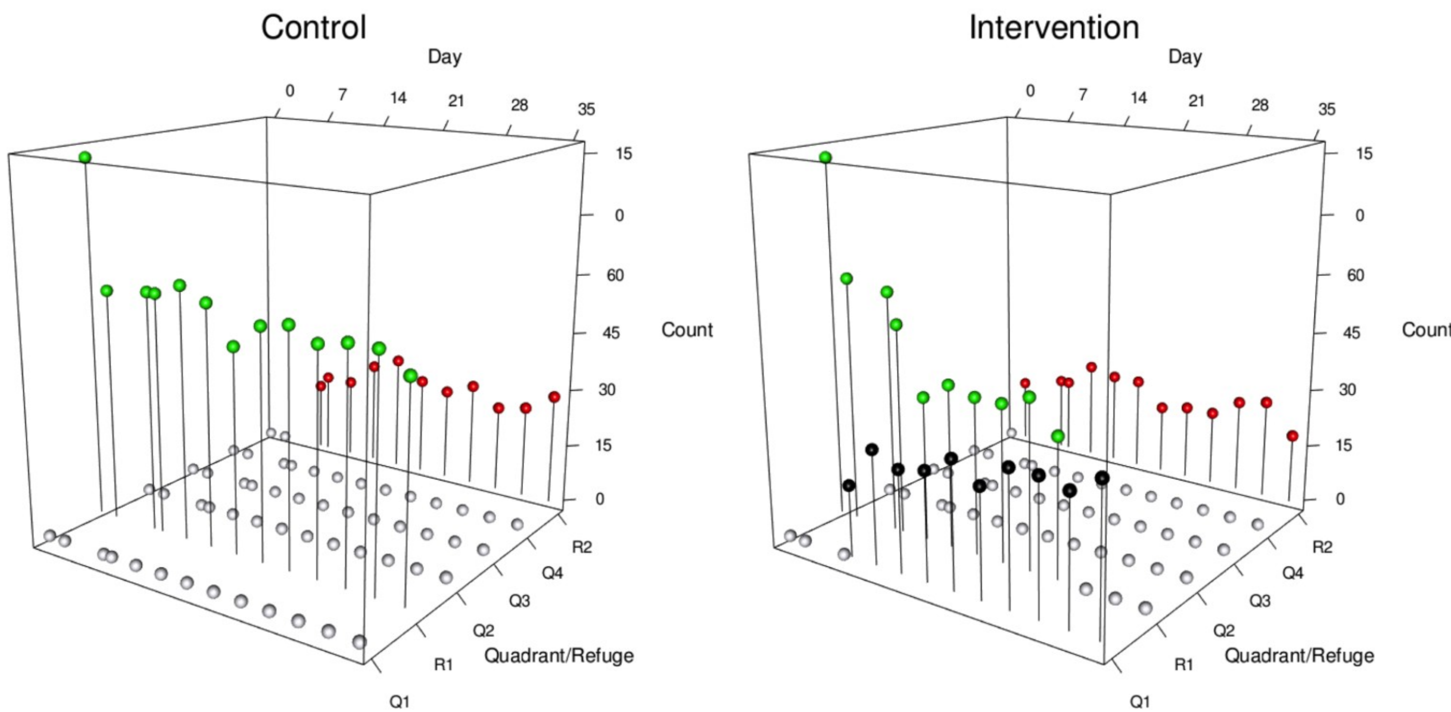
	Poisson model			**Poisson with random intercept**			**Poisson with random intercept and slope**		
	Coef.	SE	p	Coef.	SE	p	Coef.	SE	p
Intercept	3.964	0.073	<0.001	3.961	0.089	<0.001	3.956	0.114	<0.001
Tank	-0.147	0.113	0.195	-0.147	0.113	0.195	-0.150	0.113	0.187
Time (days)	-0.011	0.003	0.001	-0.011	0.003	0.001	-0.010	0.004	0.011
Tank*Time	-0.011	0.005	0.029	-0.011	0.005	0.029	-0.011	0.005	0.036
AIC	*361.85*			***31.28***			*45.76*		

Figure 3.1. Diagram (not to scale) of experimental design for each glass tank.



Video cameras recording each area of the tank (A); primary (B) and secondary (C) refuges; glass-walled maze (D); and guinea pig cage (E).

Figure 3.2. Distribution of triatomine insects on tanks over time in one of the three repetitions



Green dots represent triatomine insects in the primary refuge (R1); red dots represent triatomine insects in the secondary refuge (R2); black dots represent triatomine insects in or under the guinea pig cage (Q1); and grey dots represent no triatomine insects. Q2, Q3, and Q4 stand for quadrants 2, 3, and 4.

Figure 3.3. Observed and predicted number of triatomine insects as a function of time and intervention

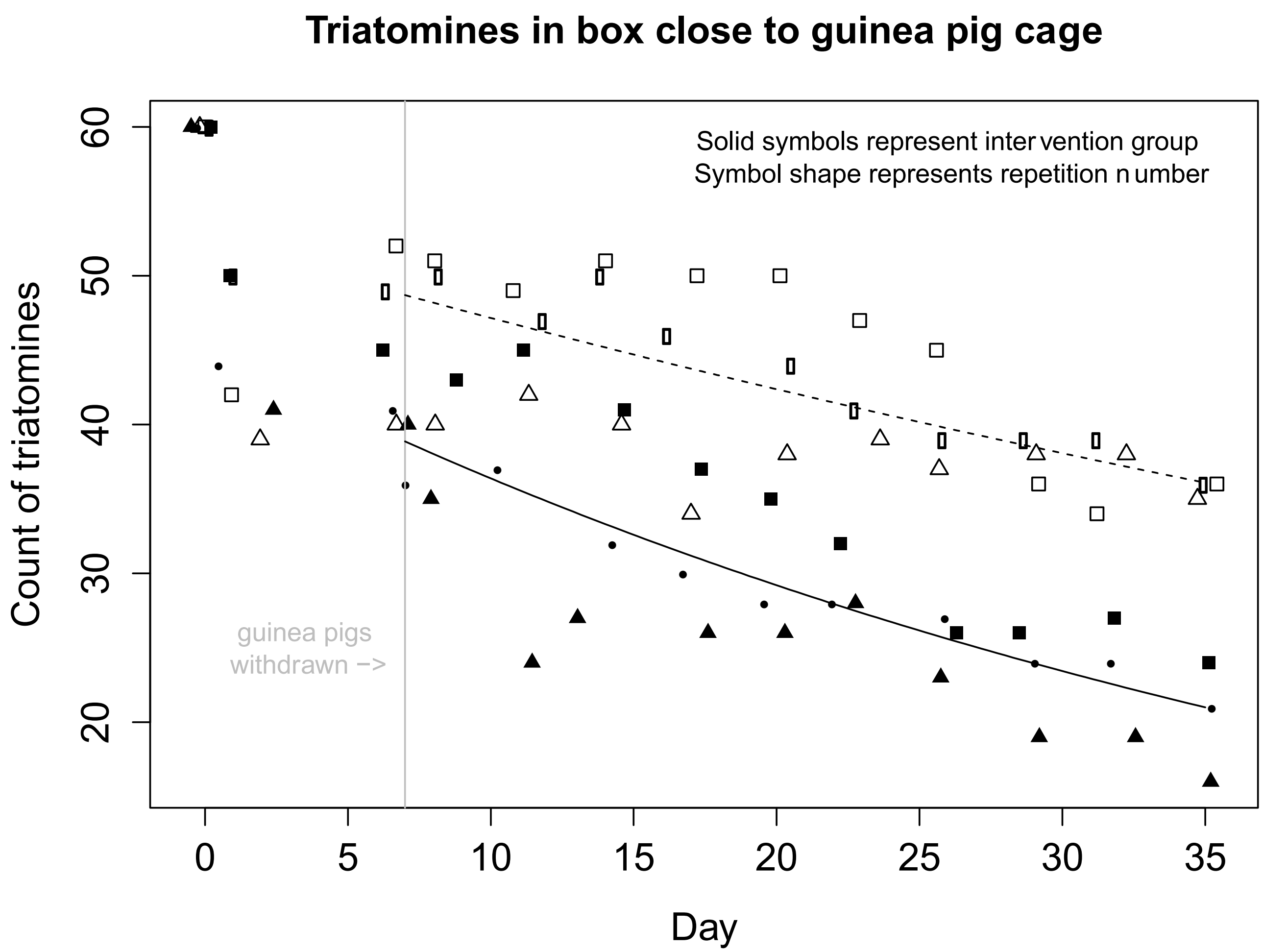
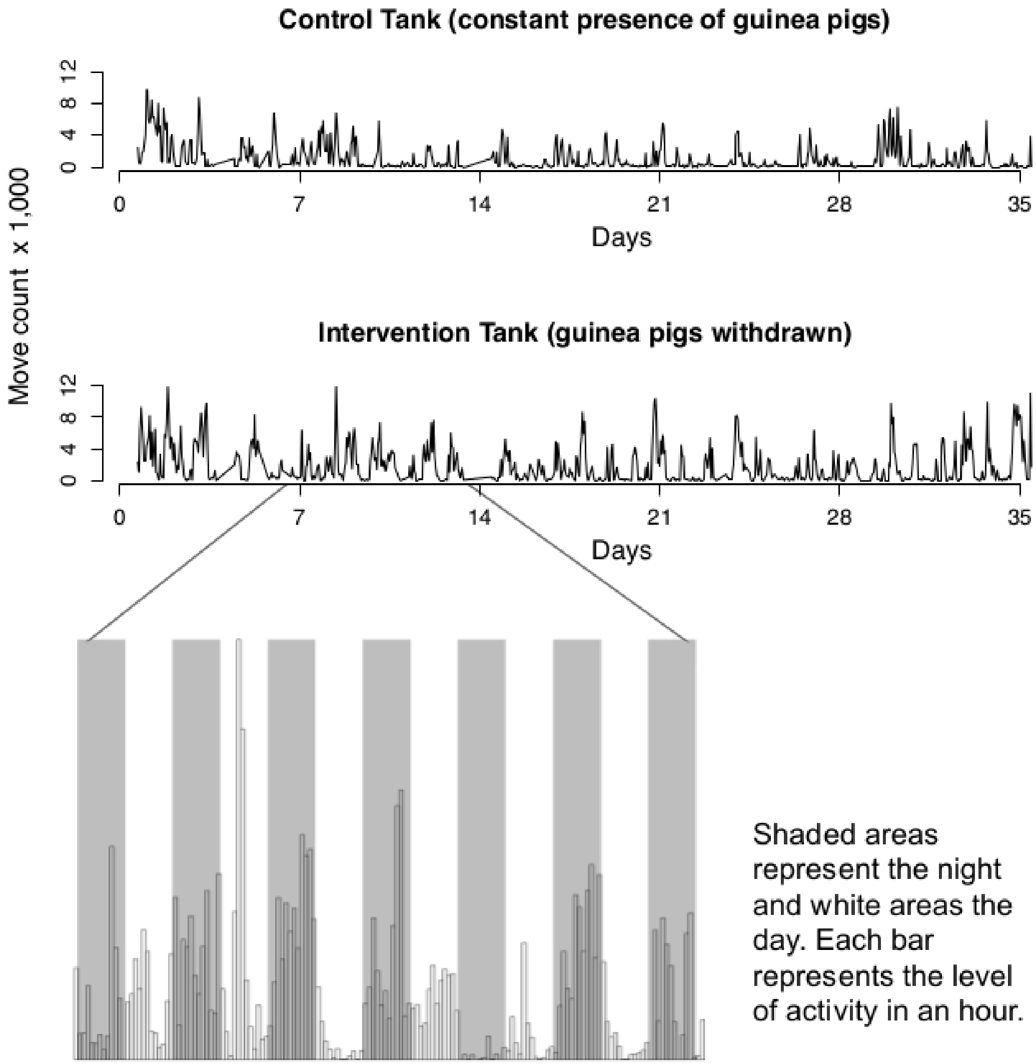
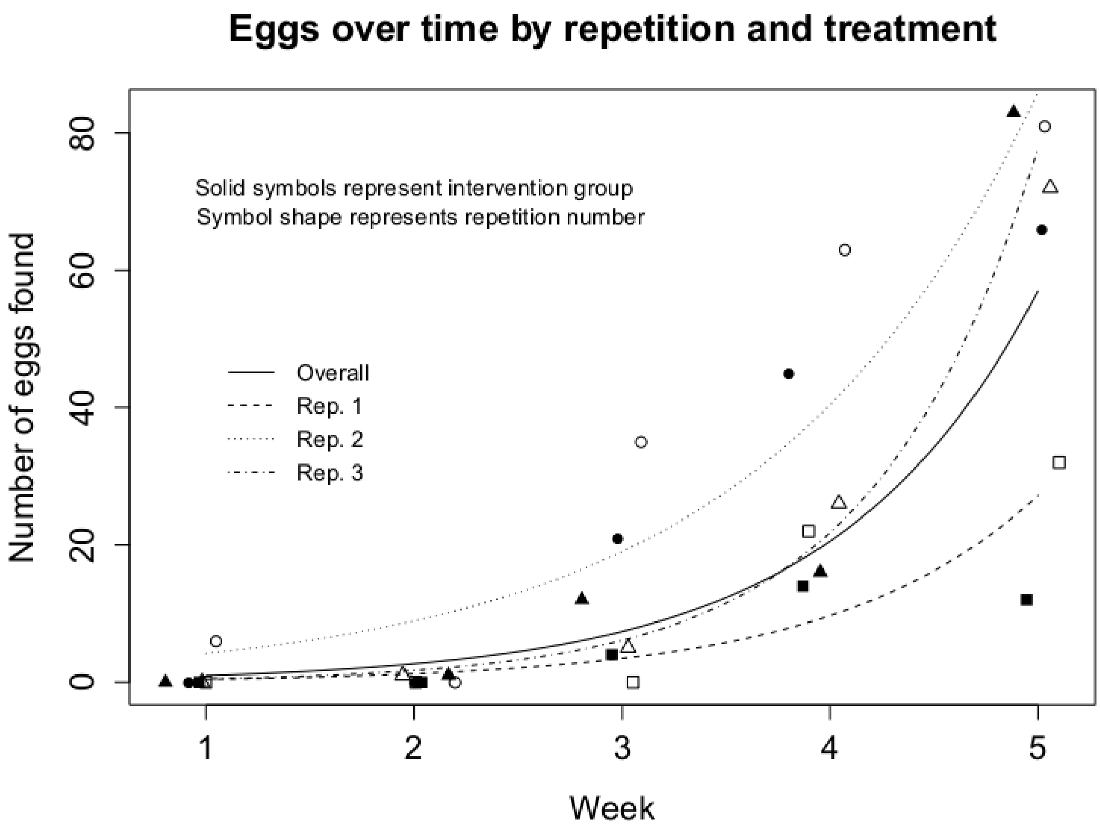


Figure 3.4. Activitiy level estimated by count of motion-activated snapshots.



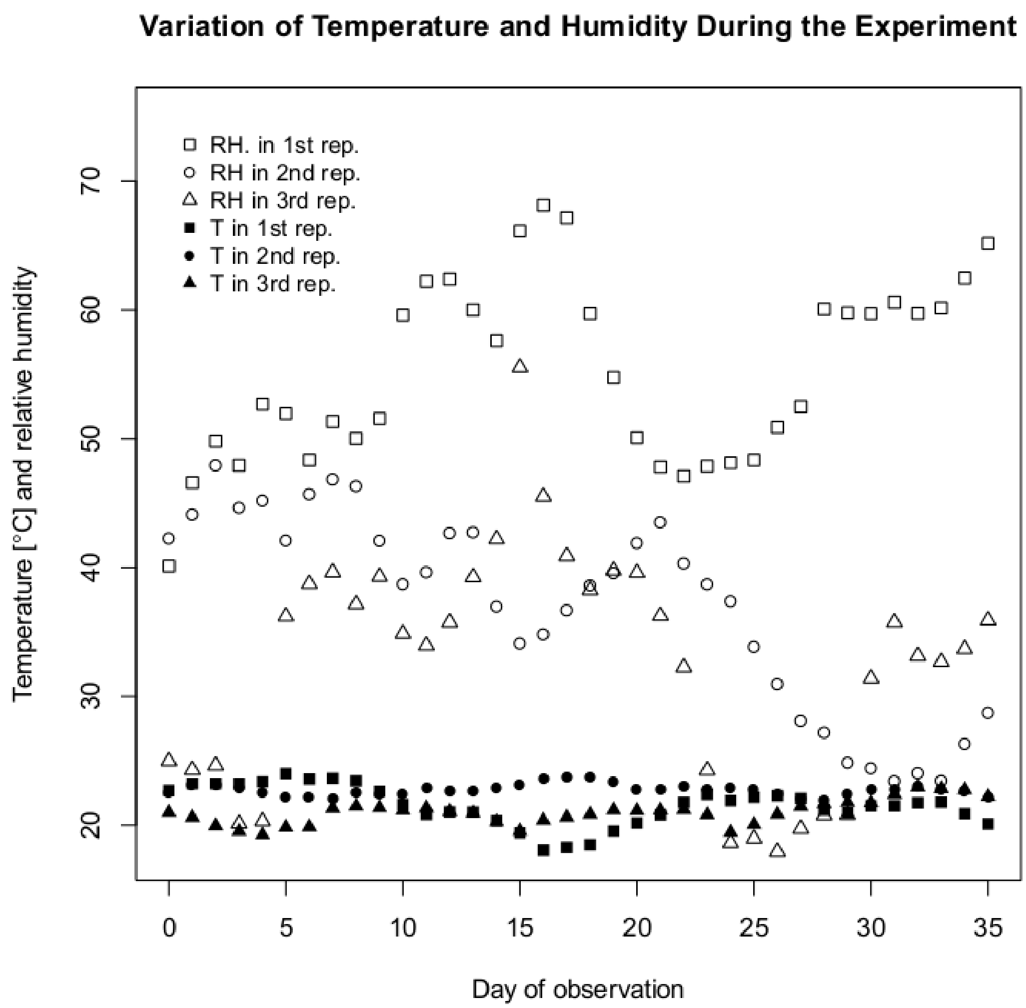
Graph shows count of moves recorded in one repetition. The inset shows a detailed histogram of moves during days and nights.

Figure 3.5. Observed and predicted number of eggs as a function of time.



Predictions based on outputs from Poisson model with random intercept and random slope.

Figure 3.6. Median daily temperature and relative humidity across repetitions.



5. Conclusions

The present dissertation thesis covers some important aspects of the ecology of the primary vector of Chagas disease in the southern cone of South America, *Triatoma infestans*. Specifically, it studies the role of domestic animals in the ecology of *Triatoma infestans*. The dissertation also examines the potential role of domestic animals, specifically dogs, as animal sentinels for the early detection of transmission of *Trypanosoma cruzi*, the causative agent of Chagas disease.

5.1. Domestic animals, their physical environment, and presence and abundance of *Triatoma infestans* and *Trypanosoma cruzi*

In the first aim of the study we found strong associations between the presence and abundance of *Triatoma infestans* and the combination of specific domestic animal species and specific construction materials used to build the animal enclosures. In particular, the presence of guinea pigs or rabbits in corrals built with stacked stones or bricks had higher odds of infestation and higher average number of captured triatomine vectors than doghouses built with mortared brick (the reference group). Guinea pigs and rabbits were also associated with increased odds of finding *Trypanosoma cruzi*-carrying insect vectors in their enclosures, and guinea pigs were also associated with a higher number of *Trypanosoma cruzi*–carrying insect vectors. Importantly, we found that 30% of the empty corrals in the area were infested with *Triatoma infestans* and 31% of these infested empty corrals had *Trypanosoma cruzi*-

infected insect vectors. Enclosures containing poultry were also associated with the odds of finding *Triatoma infestans*. In other countries dogs have been reported as important hosts for *Triatoma infestans* and as reservoir of *Trypanosoma cruzi*; however, we found that in Peru, other species might play a more important role for the presence and abundance of triatomine vectors. Currently, the Ministry of Health, through their Chagas Disease Control Program, conducts surveillance of houses for the presence of *Triatoma infestans*. Such surveillance activity should focus especially on the corrals of domestic animals, with special attention to those corrals built with stacked stones and bricks and containing small animals (guinea pigs, rabbits, and poultry). Also, surveillance programs should include observing empty animal enclosures. Interventions to reduce and control vector populations should focus on improving animal corral materials, and reducing or eliminating animals populations from the peridomiciliary area after triatomine vectors are eliminated.

5.2. Dogs and their potential as animal sentinels to detect areas of transmission of *Trypanosoma cruzi*

In the second aim of this dissertation we found a strong spatial association between canine seropositivity for *Trypanosoma cruzi* and *Trypanosoma cruzi*-infected vector colonies. All seropositive dogs in the study area were 9 months or older and the odds for a dog to be seropositive increased with age. In the same study area we did not find any seropositive humans under 5 years of age and only 5 seropositive humans under 18 out of 513 sampled humans. Finally, we found that 97% of all the

T. cruzi-infected insect vectors were captured outside de houses. Taken together, this constellation of findings suggest that at the time of our study, the insect vectors and the parasite *T. cruzi* had begun transmission among the canine population but did had not yet become established in the human population. Dogs that lived within 50 meters of infected vector colonies had 5.8 times higher odds of being seropositive compared to dogs living further. Dogs have the potential of being animal sentinels to detect early transmission of *Trypanosoma cruzi*. In Argentina dogs were disregarded as sentinels because their behavior is such that they do not represent the *T. cruzi* transmission risk of a specific area inhabited by humans. In Argentina, dogs are usually used as hunting hounds and in addition to being infected by vectorial transmission, they may become infected by biting or eating infected wild animals. In contrast to the Argentine setting, dogs in Arequipa, Peru do not hunt, eliminating the risk of oral transmission from eating infected animals far from the area under surveillance. Dogs in Arequipa do not migrate from their homes, which reduces the probability of false positives (defined in this case as an area defined as positive by the presence of seropositive dogs that were infected outside of the area of interest for human transmission risk).

Dogs can and do eat triatomine insects, and the oral route is an important component of the transmission of *T. cruzi* in dogs. This means that dogs are at risk of infection by vectorial transmission and by the oral route, so their likelihood of exposure to *T. cruzi* is higher than in humans.

In Peru and in other Latin American countries there are health programs that already involve dogs into which a Chagas sentinel program might be embedded. In particular, anti-rabies campaigns are common in many countries in Latin America and they congregate most dogs in a few locations to administer anti-rabies shots. This is a window of opportunity to collect canine blood sample, record the age of dogs and their address and use this information to determine areas of *T. cruzi* transmission.

5.3. *Triatoma infestans* behavior and dispersion under the constant presence and removal of hosts

In the third aim of the dissertation we studied the dispersion and host-seeking behavior of *Triatoma infestans*. We found that *Triatoma infestans* displays a high level of dispersion even in the constant presence of animal hosts and that the pattern of dispersion under conditions of constant presence of hosts appears to be random. When hosts are removed, the level of activity and dispersion increases significantly, and the pattern is not random. Some of the triatomine vectors migrate closer to the original location of removed hosts and some of the triatomine vectors migrate in the opposite direction. There are important implications for these observations. First, the high level of activity and dispersion even in the constant presence of hosts means that infested animal corrals in the field represent a constant and continuous risk for humans in the area. Second, the increased activity and dispersion after hosts are removed suggest that the population dynamics of hosts in the field might increase the risk of *T. cruzi* transmission for humans. For example, when humans remove domestic livestock from enclosures to sell or for their own consumption, the vector dispersal pattern and increased activity may bring vectors closer to potential human hosts. Third, the observed vector migration towards the original location of hosts indicates that empty animal corrals might remain attractive to triatomine vectors as hiding places or feeding areas for the near future.

5.4. Chagas disease: a complex and context-dependent system

Chagas disease is a very complex disease that involves dozens of triatomine species as vectors, many mammal species as reservoirs of the parasite, lifelong infections in humans, and difficulty to diagnose and treat the disease. Numerous subsystems comprised by the interactions between vectors, animal reservoirs and humans could be defined across The Americas. In this dissertation we focused on some aspects of the ecology of *Triatoma infestans* and showed that the study of domestic animals might enlighten our understanding of vector abundance and behavior, and the detection of early transmission of *Trypanosoma cruzi* in a given area. Interventions based on domestic animals might complement current vector control programs against Chagas disease and reduce the burden of this neglected tropical disease in The Americas.

Curriculum Vita

Ricardo Castillo Neyra

Personal Information

Address: 2820 Maryland Ave., Baltimore, 21218 MD

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Nationality: Peruvian

Date of Birth: March 7th, 1977

Profile

A scholar of infectious disease epidemiology with a strong background in quantitative methods, zoonotic diseases, and the study of complex systems. Well versed in designing and conducting community-based studies. Strong data management and analysis skills; experience with a variety of data sources. A team player that enjoys working with people with different backgrounds, and learning quickly.

Education

2014 **Doctor of Philosophy (Ph.D.) in Epidemiology**

Johns Hopkins Bloomberg School of Public Health (JHBSPH), Baltimore, MD, USA.

Dissertation title: "Role of domestic animals in the small-scale ecology of *Triatoma infestans*, a vector of Chagas disease"

2014 **Master of Science in Public Health (MSPH) in International Health**

JHBSPH, Baltimore, MD, USA.

2007 **Doctor of Veterinary Medicine (D.V.M)**

San Marcos Major National University (SMMNU), Lima, Peru.

Dissertation title: " Spatial relationship between human taeniasis by *Taenia solium* and parasitic burden of porcine cysticercosis"

2006 **Bachelor of Science (B.S.) in Veterinary Medicine**

San Marcos Major National University. Lima, Peru.

Research Experience

2011 - 2014 **Student Investigator**. Environmental Health Sciences Department, JHBSPH

Project title: "Reducing exposures to pathogens from Industrial Food Animal Production". (NIOSH -funded research project).

Designed methods to collect field data, conducted field surveys, conducted statistical analyses, and wrote scientific articles and posters.

2008 – 2009 **Project Coordinator**. Cayetano Heredia University, Peru.

Project title: "Eco-epidemiology of Chagas' Disease in Southern Peru" (NIH/TMRC-funded research project).

Managed a team of 21 persons to lead several field and lab-based studies under an NIH grant to investigate improved strategies to control the insect vector of Chagas disease. Coordinated activities with local community leaders and the MoH.

2008 - 2009 **Coordinator of Veterinary Studies**. Cayetano Heredia University, Peru.

Project title: "Eco-epidemiology of Chagas' Disease in Southern Peru" (NIH/TMRC-funded research project).

Designed and conducted community-based studies, including a domestic animal serology survey and spatial mapping to look for hotposts of *T. cruzi.* Oversaw laboratory activities involving animal models to study Chagas disease.

2006 - 2008 **Coordinator of Field Work**. Laboratory of Preventive Veterinary Medicine, San Marcos Major National University, Peru.

Project title: "Systematic study of the environmental and public health impact of the porcine industry" (CLF-JHBSPH-funded research project).

Conducted extensive microbiological testing of bacterial pathogens in pig feces, collected spatial data, and organized all logistics for the field and laboratory work.

2005 - 2008 **GIS Manager.** Laboratory of Preventive Veterinary Medicine, San Marcos Major National University, Peru.

Project title: "A demonstration project to eliminate cysticercosis in Peru, a model by which the disease can be eradicated in other parts of the world" (Melinda & Bill Gates Foundation - funded research project).

Collected spatial data, created a GIS to organize fieldwork, conducted spatial analyses to study the epidemiology of cysticercosis in pigs.

2006 - 2007 **Research Assistant**. Laboratory of Preventive Veterinary Medicine, San Marcos Major National University, Peru.

Project title: "Eradication of *Echinococcus granulosus* by targeting dog to human transmission" (NIAID-funded research project).

Determined the burden of positive animals in different models of experimental infections, and collected human data in endemic areas.

Teaching Experience

2010 - 2012 **Lead Teaching Assistant.** Course Title: Principles of Epidemiology. Department of Epidemiology, JHBSPH. Led instructors' meetings, developed and graded exams, and guided new TAs during lab discussion (~300 graduate students).

2012 **Invited Lecturer**. Lecture Title: "American Trypanosomiasis". Course Title: Zoonosis. SMMNU, Peru (Undergraduate students).

2012 **Teaching Assistant**. Course Title: Fundamentals of Epidemiology. Undergraduate Program in Public Health Studies, JHU. Led weekly discussions as the sole section instructor, graded homework, and held office hours (~30 undergraduate students).

2012 **Teaching Assistant**. Course Title: Data Analysis Workshops I & II. Department of Biostatistics, JHBSPH. Attended all class sessions in this intense computer lab-based workshop to assist students with hands-on learning in the use of statistical computing packages and answer inquiries related to statistical analyses. Reviewed homework assignments (~55 graduate students).

2010 **Teaching Assistant**. Course Title: Epidemiologic Methods III. Department of Epidemiology, JHBSPH. Led laboratory discussions, held office hours, and graded homework and exams (~60 graduate students).

2008	**Invited Lecturer**. Lecture Title: "Principles of Spatial-veterinary epidemiology". Course Title: Veterinary Epidemiology. SMMNU, Peru. (Undergraduate students).
2007	**Teaching Assistant**. Course Title: Mathematics & Statistics. SMMNU, Peru. Led practice laboratories, developed and graded homework and exams (~80 undergraduate students).

Peer Reviewed Publications

Castillo Neyra R, Chou Chu L, Llamosas Chu M, Levy MZ. (In preparation). Spatial association between *Trypanosoma cruzi* seropositive dogs, guineas pigs and *Triatoma infestans*.

Castillo Neyra R, Barbu C, Salazar R, Borrini K, Naquira C, Levy MZ. (Under review in PLoS NTD). Host-seeking behavior and dispersal of *Triatoma infestans*, a vector of Chagas disease, under controlled conditions.

Castillo Neyra R, Frisancho AJ, Resnick C, Carroll KC, Rinsky JL, Rule AM, Silbergeld EK. 2014. Multidrug-Resistant and Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Hog Slaughter and Processing Plant Workers and Their Community in North Carolina (USA). Environmental Health Perspectives. http://ehp.niehs.nih.gov/1306741/

César M. Jayashi, Armando E. Gonzalez, **Ricardo Castillo Neyra**, Silvia Rodríguez, Hector H. García, Marshall W. Lightowlers, and the Cysticercosis Working Group in Peru. 2013. Validity of the Enzyme-linked Immunoelectrotransfer Blot (EITB) for naturally acquired porcine cysticercosis. Veterinary Parasitology. http://www.sciencedirect.com/science/article/pii/S0304401713005645

Jayashi CM, Gonzalez AE, **Castillo Neyra R**, Kyngdon CT, Gauci CG, Lightowlers MW. 2012. Characterisation of antibody responses in pigs induced by recombinant oncosphere antigens from *Taenia solium*. Vaccine. Dec; 30(52): 7475-80. http://www.ncbi.nlm.nih.gov/pubmed/23116696

Castillo Neyra R, Davis MF, Vegonsen L, Price LB, Silbergeld EK. 2012. Antimicrobial-resistant bacteria: an unrecognized work-related risk in food animal production. Saf Health Work. Jun; 3(2):85-91. http://www.e-shaw.net/article/S2093-7911(12)32001-5/fulltext

Tustin A, Small D, Delgado S, **Castillo Neyra R**, Verastegui M, Ancca Juarez JM, Quispe Machaca VR, Gilman R, Bern C, Levy MZ. 2012. Use of individual level covariates to improve latent class analysis of *Trypanosoma cruzi* diagnostic tests. Epidemiologic Methods. 1(1):33-54. http://www.degruyter.com/view/j/em.2012.1.issue-1/2161-962X.1005/2161-962X.1005.xml

Hunter G, Ancca J, Verastegui M, **Castillo Neyra R**, Borrinini K, Náquira C, Cornejo JG, Málaga F, Cordova E, Gilman RH, Bern C, Levy MZ. (2012) A Field Trial of Alternative Targeted Screening Strategies for Chagas Disease in Arequipa, Peru. PloS Negl Trop Dis 6(1): e1468. http://www.plosntds.org/article/info%3Adoi%2F10.1371%2Fjournal.pntd.0001468

Delgado S, **Castillo Neyra R**, Quispe VR, Ancca J, Chou L, Verastegui M, Bocángel C, Tustin AW, Sterling C, Comrie A, Náquira C, Cornejo JG, Gilman RH, Bern C, Levy MZ. (2011) A History of Chagas Disease Transmission, Control, and Re-Emergence in Peri-Rural La Joya, Peru. PloS Negl Trop Dis 5(2): e970. http://www.plosntds.org/article/info%3Adoi%2F10.1371%2Fjournal.pntd.0000970

Presentations (first author or presenter)

2014	"Dispersion and behavior of *Triatoma infestans* (a kissing bug) when blood meal sources are removed". Invited Speaker. Disease Modeling Group. Department of Ecology and Evolutionary Biology Princeton University, USA.
2014	"Impact of host removal on dispersal of *Triatoma infesta*ns, a vector of Chagas disease". Ecology and Evolution of Infectious Diseases. 12th Annual Conference. Fort Collins, CO, USA.
2013	"Spatial association between *Trypanosoma cruzi* seropositive dogs and *Triatoma infestans".* The American Society of Tropical Medicine & Hygiene. 62nd Annual Meeting. Washington, DC, USA.
2013	"Workers on the front line: pathogen exposures and injuries in swine slaughter and processing". National Environmental Health Association – Annual Educational Conference & Exhibition. Washington, DC, USA.
2012	"Guinea pigs and the Epidemiology of Chagas Disease: Implications for control". First International Conservation Medicine Symposium. Lima, Peru.
2012	"Exposures of hog slaughterhouse workers and household members to *S. aureus* and MRSA". International Society for Environmental Epidemiology Conference. Columbia, SC, USA.
2012	"Community/Worker Exposures to Pathogens from Industrial Food Animal Production". Partnerships for Environmental Public Health Annual Meeting. NIEHS. Washington, DC, USA.

2011 "Proximity between dogs and *Trypanosoma cruzi* infected triatomines as a risk for the persistence of Chagas disease". The American Society of Tropical Medicine & Hygiene 60th Annual Meeting. Philadelphia, PA, USA.

2009 "Risk factors for *T. infestans* and *T. cruzi* presence in an endemic rural community of Arequipa, Peru". International Symposium on the Centenary of Chagas Disease. Rio de Janeiro, Brazil.

Academic Honors and Awards

- Global Health Field Research Award, Center For Global Health, Johns Hopkins University — 2013
- Baker, Reinke, Taylor Scholarship in International Health, JHBSPH — 2013
- Trainee: "Veterinarian Training in Zoonotic Disease in Developing Countries". Fogarty International Center Training Grant- NIH. — 2012
- Delta Omega Scholarship. JHBSPH — 2012
- Ellen B. Gold Award for academic achievement. Department of Epidemiology, JHBSPH — 2011
- Farming for the Future Fellowship. Center for a Livable Future, JHBSPH. — 2011
- Fulbright Scholarship. Department of State, USA. — 2009 – 11
- Farming for the Future Fellowship. Center for a Livable Future, JHBSPH. — 2010
- Miriam Brailey Award for academic achievement. Department of Epidemiology, JHBSPH. — 2010
- Farming for the Future Fellowship. Center for a Livable Future, JHBSPH. — 2009
- Bazalar Award for the best student research project in veterinary public health. SMMNU. — 2004

Affiliations/Memberships

- American Association of Public Health Veterinarians 2013 - Present
- Society for Epidemiologic Research 2013 - Present
- Tropical Medicine Dinner Club - JHBSPH 2012 - Present

Special Skills

- **Languages**: Fluent in English and Spanish (native tongue).
- **Computers**: R, STATA, GIS (ArcGIS & QGIS), Microsoft Office Suite including Access.